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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

INTERNATIONAL CENTRE FOR SCIENCE
AND HIGH TECHNOLOGY
Area Science Park, Building L2, Padriciano, 99 - 34012 Trieste, Italy
Tel.: +39-040-9228108, Fax: +39-040-9228136, http://www.ics.trieste.it

FINAL REPORT

Training Course on "Research Strategies on Medicinal and Aromatic Plants"

Bangkok, Thailand

14 - 18 August 2000

organized by

ICS-UNIDO

in collaboration with the

Research and Development Institute
Government Pharmaceutical Organization
Bangkok, Thailand

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CONTENT

Training Course on "Research Strategies on Medicinal and Aromatic Plants"

Summary

Medicinal plants have been the source of great discoveries in the medical world to revolutionize the arsenal of fighting human diseases. The role of traditional medicines in primary health care has repeatedly been emphasized at different forums by major health-care bodies. Recent trend of increasing use of natural products in developed societies, whereas has created more scope for the utilization of medicinal plants but has also added new dimensions to the problem of global conservation of medicinal plant resources. Irreparable loss from disappearing undocumented knowledge of treating with herbs, and extinction of herbs as a result of human activities and over-harvesting have generated fears among the conservationists. This calls for steps to document unrecorded traditions and explore their potential to discover useful drugs, which could provide foundation for the conservation of plants, which are medicinally important.

Between 35,000-70,000 plant species are used worldwide for medical purposes, but no more than 100 plant species are used to obtain clinically important drug molecules. A very small portion representing less than 10% of over 250,000 plant species has been screened in different laboratories to determine their therapeutic potential. The situation demands increased emphasis on research on plant derived drugs especially in context to underdeveloped nations, which are rich in traditions, and in resources of medicinal plants.

The health impact of medicinal and aromatic plants on the basic needs of developing countries is highly important. Also international utilization of herbal medicines is in its golden days. Worldwide trend at consumer level favors the use of natural drugs, which people consider more harmonious with the human metabolism, especially to treat nutritional imbalances and chronic conditions where modern medicine have little to offer. This trend is evident from greater increase in annual growth of herbal medicines in comparison to pharmaceutical products. Discoveries of important drug molecules from medicinal plants in recent years have further strengthened the interest in traditional remedies. Ever since the Chiang Mai declaration of 1988 highlighted the vital role of medicinal plants in primary health care and their potential in new drug discovery, a number of states have initiated programmes to utilize potential of medicinal plants in modern day therapeutics. Efforts of international bodies like WHO, FAO, UNIDO, WWF, ESCOP have provided impetus to research and development of drugs from medicinal plants. The issue is more relevant to countries, which lack economic resources but have strong traditions of using medicinal herbs in health maintenance and are rich in biodiversity. This Training Course is in accordance with a resolution made at Chiang Mai, urging UN agencies to work for improving utilization, conservation and exploitation of medicinal plants for the benefit of global community. In this context, it is planned to invite researchers working in the area of medicinal plants to interact and upgrade their knowledge for the benefit of their states.

The training course will provide opportunity to the participants from developing countries to interact with experts to know of global trends in research on medical plants. The platform will be used to train young researchers on research strategies that are best suited to the needs of developing countries. The sharing of knowledge, information and state of the art research methodologies in medicinal plant research will help to define directions for the benefit of individual states. Interaction at the training course will provide opportunities for collaborative research for mutual benefit.

Recommendations

The important role of herbal medicine in primary health care and their potential in new drug discovery is now widely recognised and many countries have initiated programmes to encourage the use of herbal medicine to complement modern medicine. In developing countries the production of herbal products has not kept pace with scientific and technological advances resulting in ill defined and poor quality products. Recognising the need to focus on the production of standardised herbal products which are effective and scientifically acceptable, ICS-UNIDO has over the past 17 years been involved in programmes designed to develop plant based medicines and health products in developing countries, in the form of seminars, workshops and technology transfer.

The R&D Institute, GPO has recently organised a training course on "Research Strategies on Medicinal and Aromatic Plants" from 14th - 18th August 2000 in collaboration with ICS-UNIDO. We would like to offer a few suggestions based on our experience in conducting the training course and feedback from the participants.

I) The ICS-UNIDO Training Courses are held annually. Each course should be devoted to one particular stage involved in the production of herbal products i.e. collection, extraction, purification/isolation, bioactivity screening, safety and clinical evaluation, quality control, documentation, regulatory affairs etc., so that the subject can be dealt with in depth and hands on practical experience can be offered to the participants.

We believe there is justification for such an approach. Participants from countries such as Laos, Cambodia, Vietnam, Bhutan, Myanma, Nepal would be the first to admit that the stage of science and technology in their respective countries is in its infancy due to various factors including limited infrastructure, shortage of funds and equipment and most important lack of well trained personnel.

The training courses organised by ICS-UNIDO during the past 3 years include the following - 1) Training Course on Industrial Exploitation of Indigenous Medicinal and Aromatic Plants held in Beijing, China, 17th - 27th June 1997.

2) Training workshops on Quality Control of Medicinal and Aromatic Plants and their products held in Jammu, India, 15th - 20th 1998.

3) Training Course on Process Simulation and Essential Oil Extraction from Aromatic Plants held in Trieste, Italy, 18th - 22nd October 1999.

The training course held in Beijing (1997) is similar to the recently concluded workshop in Bangkok in that both courses dealt with several stages of the production process. The courses held in Jammu (1998) and Trieste (1999) are more circumscribed, concentrating on quality control and process simulation and aromatic oil extraction respectively. One colleague who attended the Trieste Workshop professed a preference for a workshop which focused on one major topic and allowed hands on practical experience.

Most of the participants at the recently concluded workshop in Bangkok indicated in the evaluation questionnaires that they thought "the students scientific knowledge was balanced". This may well be true in terms of basic scientific knowledge as pointed out by one participant but another observed that the scientific knowledge of the participants was unbalanced due to the varied background. This could be nearer the truth since the workshop covered a wide spectrum of topics which in turn has attracted people working in different specialties. Two participants have proposed that the training workshops should be more focused. One has suggested that

the ICS-UNIDO should hold "a series of courses on medicinal plants in which the first course is an overview, the second on one specific topic i.e. QC, the third on another topic and so on."

Bearing in mind the lack of adequately trained personnel in developing countries we feel that importance should be attached to the "training of trainers" and this can only be done if hands on practical experience are provided in addition to theoretical knowledge. Half of the participants have indicated that they want the training/laboratory sessions (preferably "hands on" rather than just demonstrations) to be expanded. At the recently concluded workshop we could only provide demonstrations due to 1) the number of topics covered 2) time constraint and 3) the number of participants (18).

II) To provide facilities for hands on practical experience the number of participants will have to be limited (depending on the available facilities).

III) The duration of the workshop may have to be increased from 5 days to 10 days. Four participants have found the period of training to be too short.

IV) If the training course is to focus on one or two topics at a time it will be necessary to attract the right kind of participants i.e. those working in the specialty or those wishing to be trained in order to start a lab in that specialty in their own institutes.

These suggestions may go some way in alleviating the shortage of trained personnel in developing countries, which apart from the shortage of funds and lack of equipment is the constraint most often given in most of the country reports.

Problems

Twenty one participants including three from Nigeria were invited to attend the ICS-UNIDO Training Course on "Research Strategies on Medicinal and Aromatic Plants" held at the R&D Institute, Government Pharmaceutical Organization, Bangkok from 14th-18th August 2000. The three participants from Nigeria were unable to attend due to unforeseen circumstances. Formal invitations to enable our Nigerian colleagues to obtain visas were issued but we were informed that Thailand no longer had diplomatic representation in Nigeria, a fact we were unaware of. On further inquiry we learnt that Nigerian citizens would not be able to obtain visas on arrival at Don Muang airport. The Thai Immigration advised that the participants from Nigeria should travel to the UK, South Africa or Malaysia and apply for visas at the respective Thai embassies, a process which would take two working days. All the bureaucracy involved and the problem of travelling to another country to obtain visas to enter Thailand would have meant a lot of unnecessary trouble for our Nigerian colleagues. In addition they would not have made it in time to attend the training course. We deeply regret the inability of our Nigerian colleagues to attend the training course due to circumstances beyond our control.

INTERNATIONAL CENTRE FOR SCIENCE
AND HIGH TECHNOLOGY
Area Science Park, Building L2, Padriciano, 99 - 34012 Trieste, Italy
Tel.: +39-040-9228108, Fax: +39-040-9228136, http://www.ics.trieste.it

AID-MEMOIRE

Training Course on "Research Strategies on Medicinal and Aromatic Plants"

Bangkok, Thailand

14 -- 18 August 2000

organized by

ICS-UNIDO

in collaboration with the

Research and Development Institute
Government Pharmaceutical Organization
Bangkok, Thailand

BACKGROUND

Medicinal plants have been the source of great discoveries in the medical world to revolutionize the arsenal of fighting human diseases. The role of traditional medicines in primary health care has repeatedly been emphasized at different forums by major health-care bodies. Recent trend of increasing use of natural products in developed societies, whereas has created more scope for the utilization of medicinal plants but has also added new dimensions to the problem of global conservation of medicinal plant resources. Irreparable loss from disappearing undocumented knowledge of treating with herbs, and extinction of herbs as a result of human activities and over-harvesting have generated fears among the conservationists. This calls for steps to document unrecorded traditions and explore their potential to discover useful drugs, which could provide foundation for the conservation of plants, which are medicinally important.

Between 35,000-70,000 plant species are used worldwide for medical purposes, but no more than 100 plant species are used to obtain clinically important drug molecules. A very small portion representing less than 10% of over 250,000 plant species has been screened in different laboratories to determine their therapeutic potential. The situation demands increased emphasis on research on plant derived drugs especially in context to underdeveloped nations, which are rich in traditions, and in resources of medicinal plants.

JUSTIFICATION

The health impact of medicinal and aromatic plants on the basic needs of developing countries is highly important. Also international utilization of herbal medicines is in its golden days. Worldwide trend at consumer level favors the use of natural drugs, which people consider more harmonious with the human metabolism, especially to treat nutritional imbalances and chronic conditions where modern medicine have little to offer. This trend is evident from greater increase in annual growth of herbal medicines in comparison to pharmaceutical products. Discoveries of important drug molecules from medicinal plants in recent years have further strengthened the interest in traditional remedies. Ever since the Chiang Mai declaration of 1988 highlighted the vital role of medicinal plants in primary health care and their potential in new drug discovery, a number of states have initiated programmes to utilize potential of medicinal plants in modern day therapeutics. Efforts of international bodies like WHO, FAO, UNIDO, WWF, ESCOP have provided impetus to research and development of drugs from medicinal plants. The issue is more relevant to countries, which lack economic resources but have strong traditions of using medicinal herbs in health maintenance and are rich in biodiversity. This Training Course is in accordance with a resolution made at Chiang Mai, urging UN agencies to work for improving utilization, conservation and exploitation of medicinal plants for the benefit of global community. In this context, it is planned to invite researchers working in the area of medicinal plants to interact and upgrade their knowledge for the benefit of their states.

OBJECTIVES

The training course will provide opportunity to the participants from developing countries to interact with experts to know of global trends in research on medicinal plants. The platform will be used to train young researchers on research strategies that are best

FINANCIAL ADMINISTRATIVE ARRANGEMENTS FOR UNIDO-ICS FINANCED PARTICIPANTS

For those who will be invited by UNIDO-ICS to participate in the training course, round-trip air-economy transportation from the airport of departure will be arranged and prepaid tickets issued where necessary by the local organizers.

Board and lodging will be covered by ICS also through the local organizers for the nights of stay in Bangkok. You will receive an amount of US$ 25 for the nights of stay in Bangkok as pocket money. Reservations will be made for all participants at the same hotel.

The participants will be required to bear the following costs:

All expenses in their home country incidental to travel abroad, including expenditures for passport, visa, and any other miscellaneous items. UNIDO-ICS will not assume responsibility for any of the following costs, which may be incurred by the participant while attending the meeting:

1. compensation for salary or related allowances during the period of the Training Course;
2. any costs incurred with respect to insurance, medical bills and hospitalization fees;
3. compensation in the event of death, disability or illness;
4. loss or damage to personal property of participants while attending the Training Course.

VISA ARRANGEMENTS

Participants are requested to arrange for their visa as early as possible at the Thailand Embassy in their home country. In case of difficulties, please advise the contact person mentioned below.

CONTACT PERSONS

For additional information, please contact:

At ICS-UNIDO, Trieste, Italy

Mrs. Elisa S. de Roa, Earth, Environmental and Marine Sciences and Technologies, ICS-UNIDO, Area Science Park, Padriciano 99, 34012 Trieste, Italy, Tel: +39-040-9228108, Fax: +39-040-9228136, E-mail: roa@ics.trieste.it.

Dr. Karan Vasisht, Scientific Consultant, Earth, Environmental and Marine Sciences and Technologies, ICS-UNIDO, Area Science Park, Padriciano 99, 34012 Trieste, Italy, Tel: +39-040-9228139, Fax: +39-040-9228136, E-mail: vasisht@ics.trieste.it.

In Bangkok, Thailand

Dr. Krisana Kraisintu, Head, Research and Development Institute, Government Pharmaceutical Organization, 75/1, Rama 6 Road, Ratchathevi, 10500 Bangkok, Thailand, Tel.: +662-2461473, Fax: + 662-2462134, E-mail: krisana@mozart.inet.co.th.

suited to the needs of developing countries. The sharing of knowledge, information and state of the art research methodologies in medicinal plant research will help to define directions for the benefit of individual states. Interaction at the training course will provide opportunities for collaborative research for mutual benefit.

EXPECTED OUTPUTS

The training course is expected to provide stimulus to research and development of drugs from medicinal plants. The sharing of working knowledge, academic interaction and exposure to industrial processes will update the participants for the benefit of their states.

STRUCTURE AND TENTATIVE PROGRAMME OF THE TRAINING COURSE

The training course will consist of lectures, round-table discussions and a visit to herbal industrial unit, on the following topics:

Bioactivity screening in medicinal plants.
Recent trends in medicinal plant research.
Safety and clinical evaluation of herbal drugs.
Documentation methods for preserving ethnomedical knowledge.
Scope of chemical modifications of natural products for modern drug development.

PARTICIPATION

The training course will be attended by young researchers and experts working in the area of medicinal plants.

DOCUMENTATION

The documents available for the training course will be:

1. Aide-Mémoire of the Training Course.
2. Programme and list of participants.
3. Lecture notes.

LANGUAGE

The Training Course will be conducted in English and no translation facilities will be available. It is expected that the participants have a good command of English.

TIME AND VENUE

The Training Course will be held at the Research and Development Institute of the Government Pharmaceutical Organization from 14-18 August 2000.

Program

Monday, 14 August 2000

09:00-09:30	Registration and Opening
09:30-10:00	Introduction to ICS activities and Training Course
10:00-10:30	Coffee break
10:30-12:00	Lecture on Extraction and Purification of Medicinal Plants by Prof. Norio Aimi
12:00-13:00	Lunch break
13:00-14:00	Lecture on Chemical Modifications of Natural Products for Modern Drug development by Prof. Norio Aimi
14:00-15:30	Practical Training on Extraction and Purification of Medicinal Plants and Chemical Modification of Natural Products
15:30-15:45	Coffee break
15:45-17:00	Continuation
18:00-20:00	Welcome reception

Tuesday, 15 August 2000

09:00-10:30	Lecture on Bioactivity-Guided Screening of Some Natural Products by Prof. Haruhiro Fujimoto
10:30-11:00	Coffee break
11:00-12:00	Continuation and Discussion
12:00-13:00	Lunch break
13:00-13:30	Visiting various sections in R&D Institute
13:30-15:00	Practical Training on Bioactivity Screening in Medicinal Plants
15:00-15:15	Coffee break
15:15-16:00	Continuation

Wednesday, 16 August 2000

09:00-10:30	Lecture on Standardization and Quality Control of Medicinal and Aromatic Plants and their Products by Dr. Krisana Kraisintu
10:30-11:00	Coffee break

11:00-12:00	Good Laboratory Practice of Herbal Medicinal Products by Dr. Krisana Kraisintu
12:00-13:00	Lunch break
13:00-14:00	Visiting Production Lines of GPO
14:00-15:00	Practical Training on Standardization and Quality Control of Medicinal and Aromatic Plants and their Products
15:00-15:15	Coffee break
15:15-16:30	Continuation

Thursday, 17 August 2000

09:00-10:30	Lecture on Documentation Methods for Preserving Ethnomedical Knowledge by Prof. S. S. Handa
10:30-11:00	Coffee break
11:00-12:00	Continuation
12:00-13:00	Lunch break
13:00-15:00	Presentation of country reports
15:00-15:15	Coffee break
15:15-16:30	Recent trends in Nutriceuticals by Mr. Vivek Dhawan

Friday, 18 August 2000

09:00-10:30	Lecture on Good Clinical Practice of Herbal Medicinal Products by Prof. Sornchai Looareesuwan
10:30-11:00	Coffee break
11:00-12:00	Continuation and discussions
12:00-13:00	Lunch break
13:00-16:30	Tour of Rice bran oil factory in Singhaburi

LIST OF

-ORGANIZER

-LECTURERS

-PARTICIPANTS

Training Course on

Research Strategies on Medicinal and Aromatic Plants

August 14-18, 2000

Bangkok, Thailand

Scientific Adviser

Name	:	Dr.Karan Vasisht
Position	:	Scientific Adviser to ICS-UNIDO
Office	:	ICS-UNIDO
Office address	:	Area Science Park, Building L2, Padriciano 99
		34012, Trieste, Italy
Tel	:	+39-040-9228108
Fax	:	+39-040-9228136
Office address		
in India	:	University Institute of Pharmaceutical Sciences,
		Panjab University, Chandigarh 160014
Tel	:	+91-172-541142
Fax	:	+91-172-541142
E-mail address	:	kvasisht@hotmail.com, vasisht@ICS.trieste.it

List of Lecturers

Name : Prof Dr.Norio Aimi

Position : Director, Chemical Analysis Center, Chiba University

Professor, Research Center of Medicinal Resourses

Office : Faculty of Pharmaceutical Sciences, Chiba University

Office address : 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Tel : 81-43-290-2903

Fax : 81-43-290-2903

E-mail address : aimi@p.chiba-u.ac.jp

Name : Assoc. Prof Dr.Haruhiro Fujimoto

Position : Associate Professor

Office : Faculty of Pharmaceutical Sciences, Chiba University

Office address : 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Tel : -

Fax : 81-43-290-3021

E-mail address : fujimoto@pharmacy.p.chiba-u.ac.jp

Name : Prof S.S. Handa

Position : Chief Consultant at R&D

Office : Zandu Pharmaceutical Works Ltd

Office address : 70 Gokhale Road South Dadar, Mumbai 400025, India

Tel : 0091 22 4307021, 4304517/18

Fax : 0091 22 437591

E-mail address : drhandal@hotmail.com

zanduho@giasbmol.vsnl.net.in

Name	:	Dr. Krisana Krisintu
Position	:	Head
Office	:	Research and Development Institute,
		Government Pharmaceutical Organization
Office address	:	75/1 Rama 6 Rd. Ratchathevi 10400
		Bangkok, Thailand
Tel	:	662-2457164
Fax	:	662-2462134
E-mail address	:	krisana@mozart.inet.co.th

Name	:	Professor Dr. Sornchai Looareesuwan
Position	:	Dean
Office	:	Faculty of Tropical Medicine, Mahidol University
Office address	:	420/6 Rajvithi Rd, Bangkok 10400, Thailand
Tel	:	662-247-1688
Fax	:	662-246-7795
E-mail address	:	tmslr@mucc.mahidol.ac.th

Name	:	Mr. Vivek Dhawan
Position	:	Managing Director
Office	:	Medicap Limited
Office address	:	384 Soi 6, Pattana 3 Road, Bangpoo Industrial Estate,
		Samutprakarn 10280, Thailand
Tel	:	(662) 7093600 Ext.101 Dir.(662) 7094505
Fax	:	(662) 3240537, 3240451
E-mail address	:	vivek@medicapltd.com

List of Participants

Name	:	Mr. Phurpa Wangchuk
Position	:	Research Officer
Office	:	Chemistry Department
Office address	:	Pharmaceutical and Research Centre
		Institute of Traditional Medicine Services, Health
Tel	:	00-975-2-325731, 324390
Fax	:	00-975-2-324215
E-mail address	:	-
Home address	:	VILL : Lichibi, Goshing Geog
		Disitt : Zhemgang, Bhutan

Name	:	Ms. Abeba Berhane Kassaye
Position	:	Chemist
Office	:	Essential Oils Research Center
Office address	:	P.O. Box 3395, Addis Ababa, Ethiopia
Tel	:	251-1-611311
Fax	:	251-1-611764
E-mail address	:	Abeba@avu.org
Home address	:	Tel 251-1-126260
		P.O.Box 30684 Addis Ababa, Ethiopia

Name	:	Ms. Berna Elya
Position	:	Lecturer
Office	:	Faculty of Science and Mathematics, Department of Pharmacy, UI
Office address	:	Department of Pharmacy, Faculty of Science and Mathematics, University of Indonesia, Kampus UI- Depok
Tel	:	62-21-7270031
Fax	:	62-21-7863433
E-mail address	:	akhmadi didik<a_didik@yahoo.com>
Home address	:	JC H.Rijin RT.05 RW.11 No. 129 Desa Tuga, Kdapa Dua, Cimanggis, depok, Indonesia
Tel	:	6221-9183589

Name	:	Ms. Monekham Sengsavang
Position	:	Officer
Office	:	Traditional Medicine Research Center
Office address	:	Traditional Medicine Research Center, Ministry of Public Health, Vientiane, Lao PDR
Tel	:	(856-21) 315693
Fax	:	(856-21) 312354
E-mail address	:	trmc@laotel.com
Home address	:	Vientiane, Lao PDR, P.O. Box 9965
Tel	:	(856-21) 251042

Name	:	Dr. Naresh Kumar Satti
Position	:	Technical Officer 'B'
Office	:	Regional Research Laboratory, Jammu
Office address	:	Natural Products Chemistry Division Regional Research Laboratory, Canal Road, Jammu-180001, India
Tel	:	91-191-549084/549051
Fax	:	91-191-548607/543829
E-mail address	:	rrlj@nde.vsnl.net.in
Home address	:	House No.64-A Last Morh, Gandhi nagar Jammu Tawi-180004, India
Tel	:	91-191-452537

Name	:	Mr. Kok Seong Lim
Position	:	Pharmacy Assistant Manager
Office	:	Department of Pharmacy, UKM Hospital
Office address	:	Jalan Tenteram, Bandar Tun Razak, Cheras 56000 Kuala Lumpur, Malaysia
Tel	:	0060-3-9703727
Fax	:	0060-3-9737151
E-mail address	:	kokslim@hotmail.com (preferable)
Home address	:	19, Jalan 15/42, Taman Sejahtera, 51200 Kuala Lumpur, Malaysia
Tel	:	0060-3-62574081

Name	:	Dr. Farzana Shaheen
Position	:	Research Officer
Office	:	HEJ Research Institute of Chemistry
Office address	:	HEJ Research Institute of Chemistry, International Center for Chemical Sciences University of Karachi, Karachi-75270, Pakistan
Tel	:	(92-21) 4990007, 4986151, 473177-78
Fax	:	(92-21) 496-3373, 496-3124
E-mail address	:	zainraa@digicom.net.pk, hejric@digicom.net.pk
Home address	:	H. No. BIII 730/82 Khurram Colony Muslim Town Service Road, Rawalpindi, Pakistan

Name	:	Dr. Ritche Manos Hao
Position	:	Project Associate
Office	:	National Drug Information Center Department of Pharmacology
Office address	:	Rm 302 3RD Floor Department Of Pharmacology Medical Annex Building, University Of The Philippines College Of Medicine #547 Pedro Gil St. Ermita, Manila Philiphines 1000
Tel	:	(632) 5218251
Fax	:	(632) 5218251
E-mail address	:	pharma_health@yahoo.com, ritche@philonline.com
Home address	:	1665-A Maria Orosa St., Malate, Manila, Philippines 1004

Name	:	Dr. Jayantha Wijayabandara
Position	:	Senior Lecturer in Pharmacy
Office	:	Department of Pharmacology and Pharmacy
Office address	:	Faculty of Medicine,
		University of Colombo,
		Kynsey Road, Colombo 08, Sri Lanka
Tel	:	+94-1-695230
Fax	:	+94-1-695230
E-mail address	:	<phrm_crnb@sit.lk>
Home address	:	356/8, Kanuwana Road, Wijesingha
		Mawatha, Homagama, Sri Lanka

Name	:	Dr. Chiranjivi Regmi
Position	:	Chief, Scientific Officer
Office	:	Royal Nepal Academy Of Science & Technology (Ronast)
Office address	:	P.O. Box. 3323
		Khumaltar, Kathmandu, Nepal
Tel	:	547714
Fax	:	-
E-mail address	:	cregmi@hotmail.com
Home address	:	Tulsipur-9, Dang, Nepal
Tel	:	630553

Name	:	Dr. Richard Protacius Ngwenya
Position	:	Medical Director
Office	:	James Mobb Immune Enhancement
Office address	:	132 Chinamano Ave
		Harare, Zimbabwe
Tel	:	263-4-725973, 727135
Fax	:	263-4-739832
E-mail address	:	JAMESMOB@AFRICAONLINE.CO.ZW
Home address	:	31 Binton Quinnington,
		Borrowdale, Harare,
		Zimbabwe Tel: 263-4-860116

Name	:	Dr. Mariane Ngoulla
Position	:	WHO/AFRO Traditional Medicine Medical Officer
Office	:	World Health Organization for African Region
Office address	:	P.O. Box Be 733, Belvedere,
		Medical Schoos, Parirenyatwa Hosp, Harare, Zimbabwe
Tel	:	(1) 407 733 9347
Fax	:	(1) 407 733 9160, 263-4-700742
E-mail address	:	ngoullam@whoafr.org
Home address	:	22 Cardinals Road, Chisipite
		Harare, Zimbabwe

Name	:	Dr. Cheng Sun Kaing
Position	:	Deputy Director
Office	:	National Centre for Traditional Medicine
Office address	:	Ministry of Health, Phnom-Penh, Cambodia.
Tel	:	(855) 12 803375
Fax	:	(855) 23 724595
E-mail address	:	-
Home address	:	N 116E. Rd. 118, Sangkat Mittapheap Khan 7 Makara, Phnom-Penh, Cambodia

Name	:	Assoc. Prof. Dr. Krisana Pootakham
Position	:	Associate Professor
Office	:	Faculty of Pharmacy, Chiang Mai University
Office address	:	Faculty of Pharmacy, Chiang Mai University Chiang Mai 50200, Thailand
Tel	:	(66)-(53)-944342-3
Fax	:	(66)-(53)-222741
E-mail address	:	pmikptkh@chiangmai.ac.th
Home address	:	same as office address

Name : Assoc. Prof. Dr. Sanan Subhadhirasakul
Position : Associate Professor, Lecturer
Office : Department of Pharmacognosy
Office address : Department of Pharmacognosy,
Faculty of Pharmaceutical Sciences,
Prince of Songkla University, Hat Yai, Songkla, 90110
Tel : 66-74-428220
Fax : 66-74-428220
E-mail address : ssanan@ratree.psu.ac.th
Home address : 71/1 Kanjanavanich Rd., Kor Hong,
Hat Yai, Songkhla, 90110

Name : Assoc. Prof. Dr. Surapote Wongyai
Position : Director
Office : Research Institute, Rangsit University
Office address : Research Institute, Rangsit University,
Paholyotin Road, Muang-Aek, Patumtani 12000, Thailand
Tel : (0662) 9972222 extn. 1479
Fax : (0662) 5339470
E-mail address : surapote@rangsit.rsu.ac.th
Home address : 39/1 Soi Taksin 11, Taksin Road Bangkok 10600, Thailand

Name : Mrs. Vanida Chanteptawan
Position : Research Scientist
Office : Research and Development Institute,
The Government Pharmaceutical Organization
Office address : 75/1, Rama VI Rd., Rajthevi
Bangkok 10400, Thailand
Tel : (662) 2460042 ext. 1013
Fax : (662) 2462134
E-mail address : Teptewan@yahoo.com
Home address : 40/792 Mu-ban Prachanivet 3,
Ngamwongwan Rd., Tha-sai, Muang
Nonthaburi 11000, Thailand
Tel : (622) 9521842

Name : Mr. Sanya Hokputsa
Position : Research Pharmacist
Office : Research and Development Institute,
The Government Pharmaceutical Organization
Office address : 75/1, Rama VI Rd., Rajthevi
Bangkok 10400, Thailand
Tel : (home) (662) 8765956, (Office) (662)2461179-85 ext. 1009
Fax : (662) 2462134
E-mail address : sanyah@health.moph.go.th
Home address : 1074/16 Soi Taksin 28 Bukkhalo,
Thonburi, Bangkok, Thailand 10600

ANNEXE A

LECTURE NOTES

&

PRACTICAL HANDOUTS

INTRODUCTION
TO
ICS ACTIVITIES

Dr. Karan Vasisht

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname : Vasisht Other name : Karan

Title (Mr, Mrs, Dr, Professor, etc) Dr.

Current Position : Scientific Adviser to ICS-UNIDO

Current Place of Work : Trieste, Italy

Educational profile:

Year	Place of Study	Qualification	Field of study
1990	Chandigarh, India	Ph.D.	Pharmacognosy
		M.Pharm.	Pharmacognosy
		B.Pharm.	Pharmacognosy

Professional Experiences :

Have worked in the area of natural products. Permanently hold a position of "Reader in Pharmacognosy" at Danjab University Chanoigarh India

Current interests :

-Documentation of medicinal plants

-Isolation of bioactive molecules from antimalarial plants

International Centre
for Science and High Technology

ICS

Autonomous Institution operating within UNIDO legal framework

International Centre
for Science and High Technology

Karan Vasisht

Scientific Adviser

Industrial Utilization of Medicinal and Aromatic plants

Earth, Environmental and Marine Sciences Area

ICS-UNIDO

Area Science Park, Padriciano 99, Building L2, 34012 Trieste, Italy

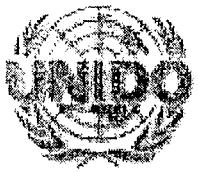
Tel: +39-040-9228106, Fax: +39-040-9228136,

E-mail: vasisht@ics.trieste.it

Objectives of ICS

- to foster and facilitate the transfer of technology in specific high-tech areas to developing countries

- to provide high-tech SMEs in developing and transition-economy countries with advanced tools and services for the enhancement of their sustainability and competitiveness



International Centre for Science and High Technology

Founded by

Nobel prize-winner Professor Abdus Salam in 1988

Supported by Italian Government

Headquarters: Trieste, Italy

(within the Area Science Park)

International Centre
for Science and High Technology

General Framework

- training courses
- scientific workshops
- high-level seminars
- fellowships
- publications and training packages


International Centre
for Science and High Technology

Cooperation with International Organizations

UNIDO

(United Nations Industrial Development Organization)

UNEP/MAP

(Mediterranean Action Plan)

MCSD

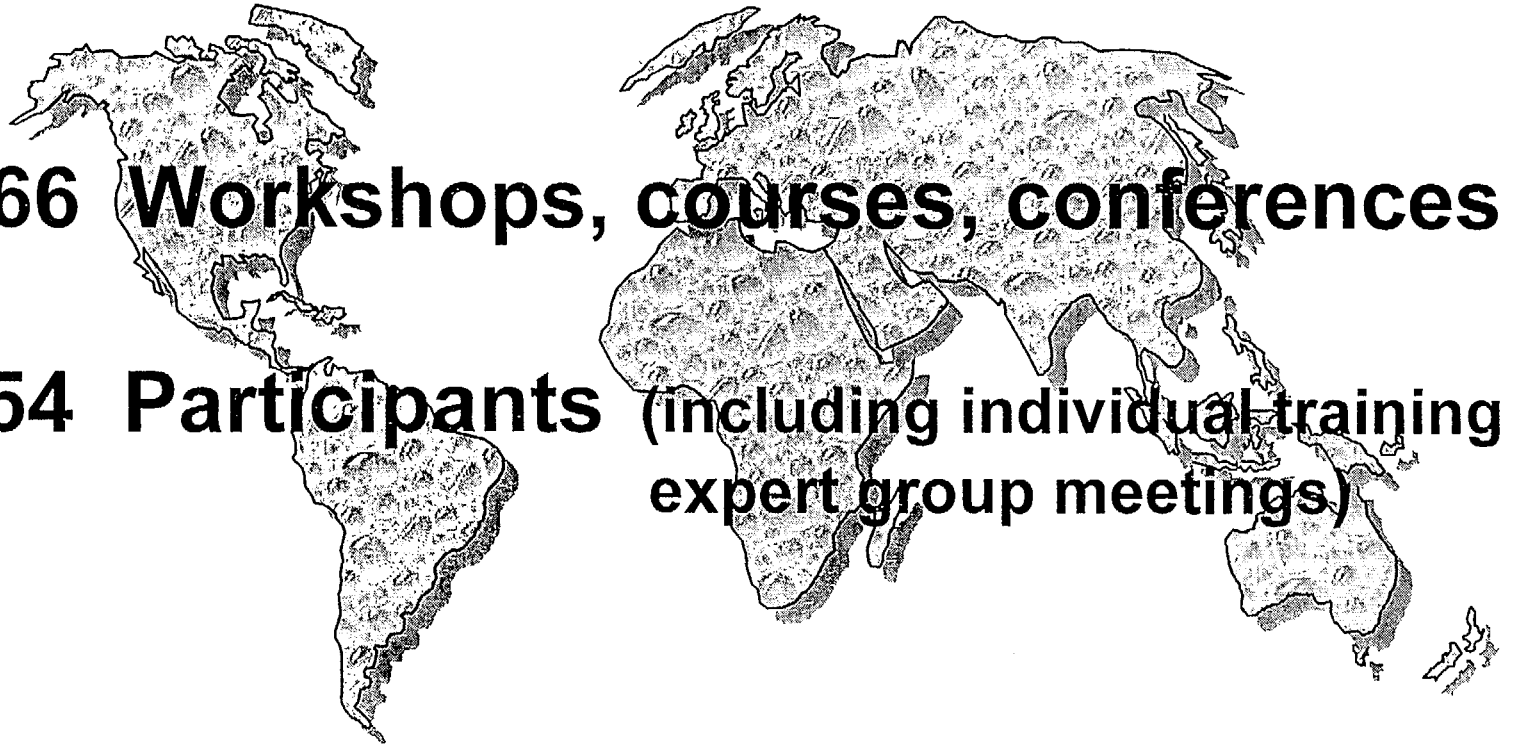
(Mediterranean Commission for Sustainable Development)

CEI

(Central European Initiative)

UNIDO ICS International Centre for Science and High Technology

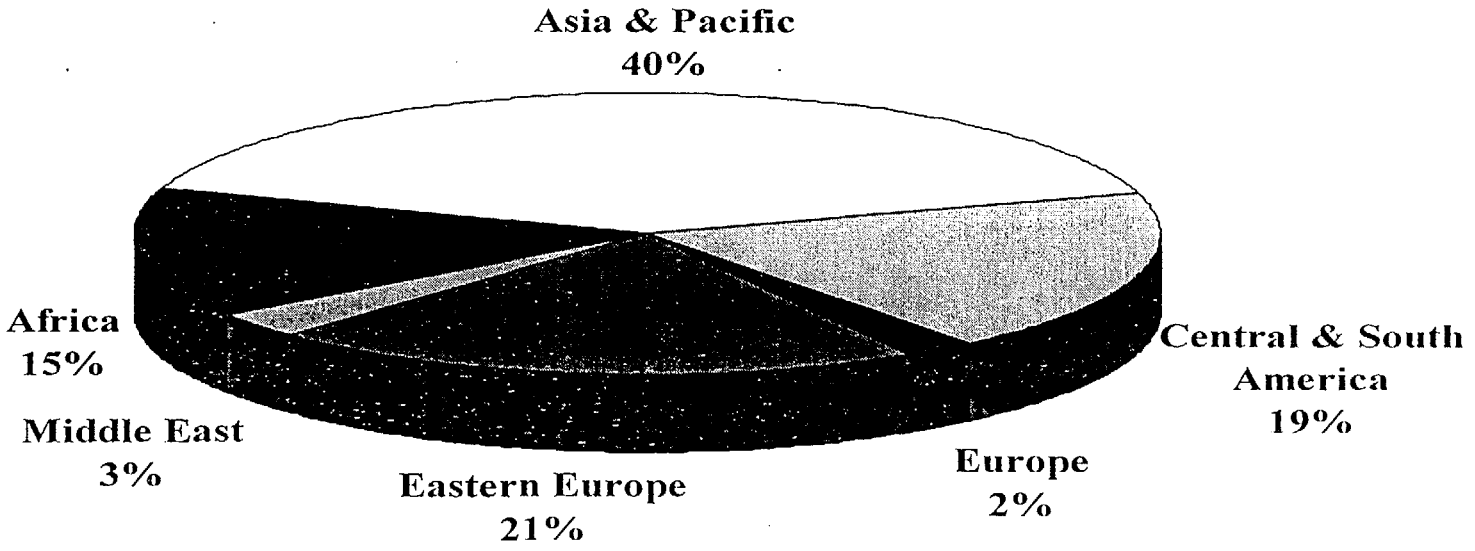
Training Activities 1988-1999



266 Workshops, courses, conferences

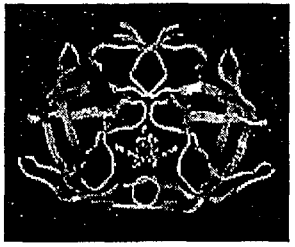
8754 Participants (including individual training & expert group meetings)

Training for Developing Countries



UNIDO ICS International Centre for Science and High Technology

Fields of Activity

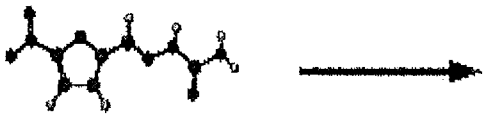


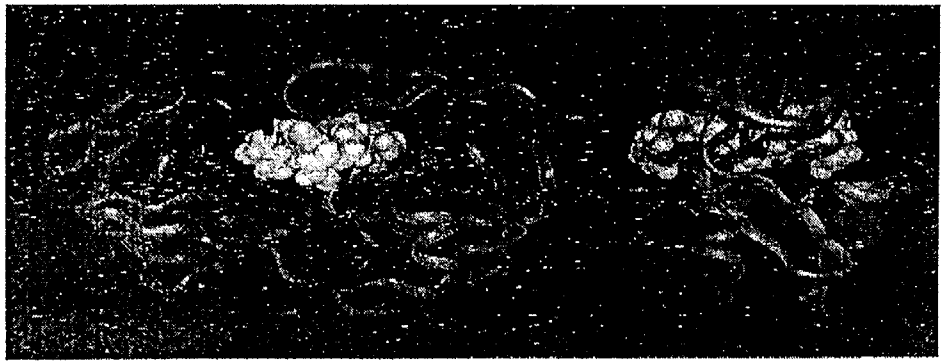
Pure and Applied Chemistry

Earth, Environmental and Marine Sciences and Technologies

High Technology and New Materials

Pure and Applied Chemistry

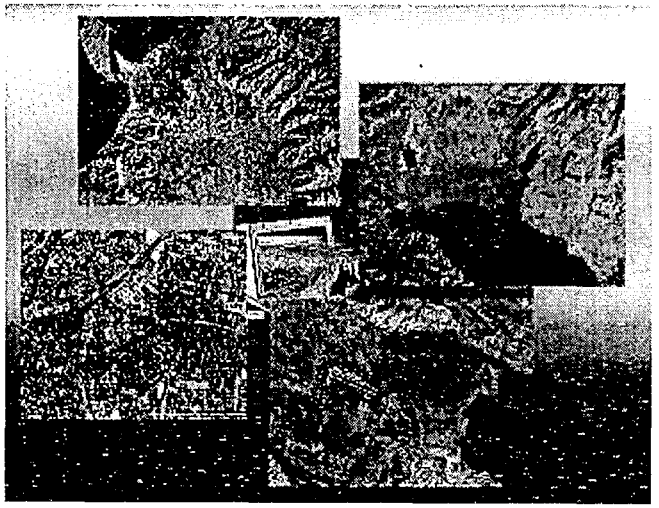
- Catalysis and sustainable chemistry
- Biodegradable plastics 
- Remediation
- Combinatorial Chemistry and Technologies



UNIDO ICS International Centre for Science and High Technology

Earth, Environmental and Marine Sciences and Technologies

- impact analysis of industrial development
- sustainable industrial exploitation of natural resources
- forecasting and monitoring
- process simulation



Environment subprogrammes

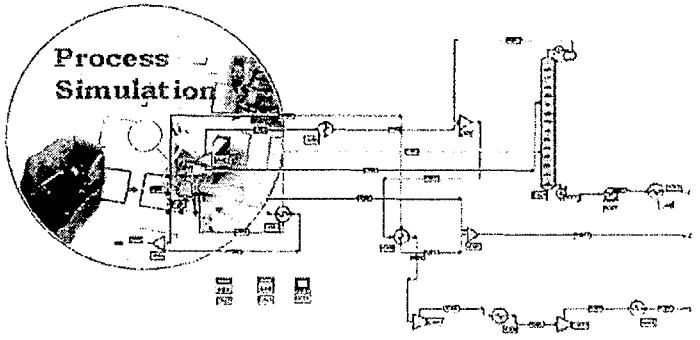
- Technologies for sustainable industrial development
- Coastal Zone Management
- Industrial Utilization of Medicinal and Aromatic Plants

UNIDO International Centre for Science and High Technology

Technologies for sustainable industrial development

- **Reinforce decision-making process for sustainable industrial development**

- **Exploit modern technical tools:**
  - **Process simulation**
  - **Remote sensing**
  - **GIS**
  - **Image processing**



Coastal Zone Management

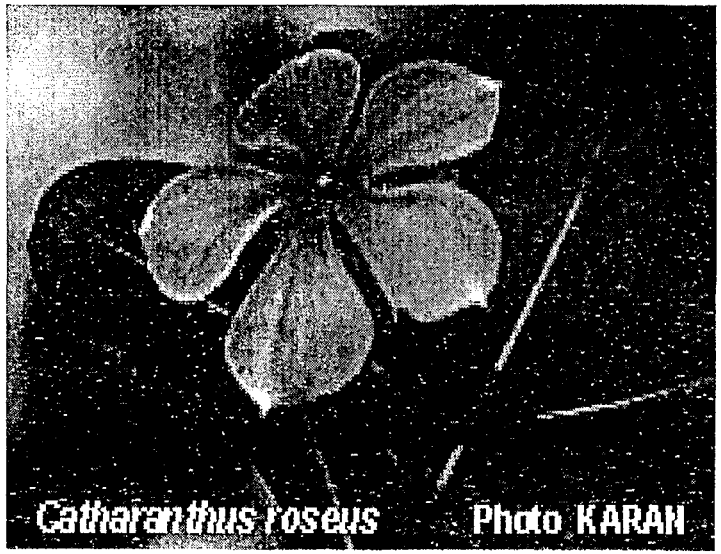
- **Sustainable development of coastal economics**
- **Integration of scientific, economic, legislative aspects**
- **Application of decision support systems for:**

  - industrial siting
  - resource management and control
  - control and monitoring of pollution
  - marine navigation control

UNIDO (ICS) International Centre for Science and High Technology

Industrial Utilization of Medicinal and Aromatic Plants

- Consolidation of existing technology for developing countries
- Technical assistance in product R&D
- Raising government awareness



High technology and New Materials

✍ high technology

laser applications and optical technologies for industry and medicine

✍ new materials

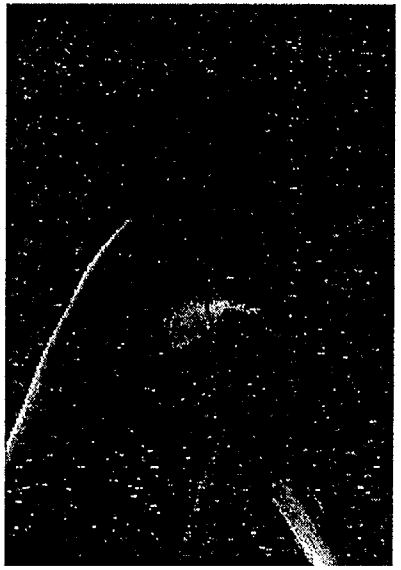
composite materials for low-cost housing

✍ photovoltaic solar energy

diffusion of pv systems and applications

✍ telecommunication technologies

radio communications, fixed, mobile, satellite and rural networks



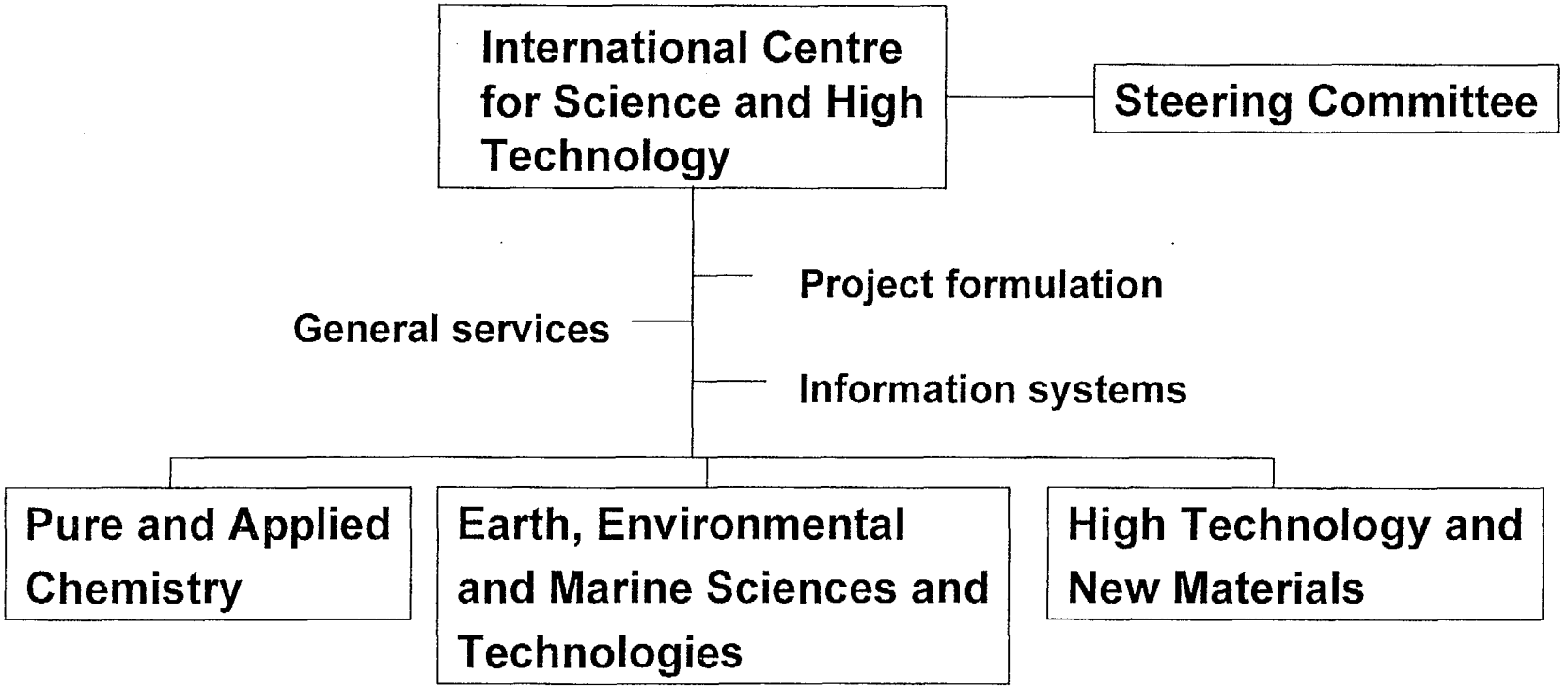
International Centre
for Science and High Technology

Networking

Identification in various regions of the world, selection and evaluation of partner institutions willing to offer

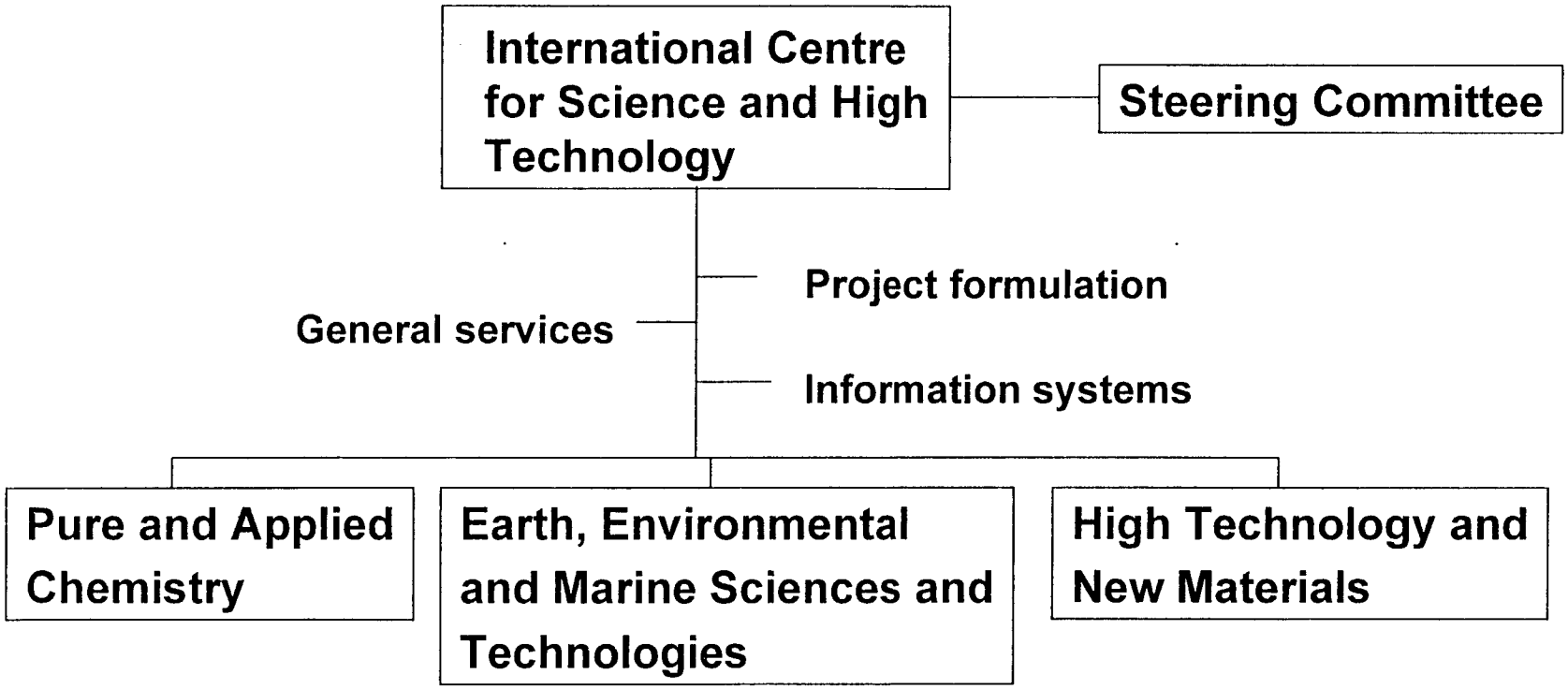
co-operation and support

Institutional Structure



UNIDO International Centre for Science and High Technology

Institutional Structure



EXTRACTION AND PURIFICATION OF MEDICINAL PLANTS

Professor Norio Aimi

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname : Norio Other name : AIMI

Title (Mr, Mrs, Dr, Professor, etc) Professor Dr.

Current Position : Director, Chemical Analysis Center, Chiba University

Current Place of Work : Faculty of Pharmaceutical Sciences, Chiba University, Japan

Educational profile:

Year	Place of Study	Qualification	Field of study
1961	The University of Tokyo	Bachelor	Pharmaceutical Sciences
1967	The University of Tokyo	Docter	Pharmaceutical Sciences

Professional Experiences :

1969-1994 Associate Professor, Faculty of Pharmaceutical Sciences, Chiba University

1997-1999 Director, Research Center of Medicinal Resources, Faculty of Pharmaceutical Sciences, Chiba University

1999- Director, Chemical Analysis Center, Chiba University

Current interests :

Structure and Activity of Natural Products Possessing Immunomodulatory and Neurotropic Activity

Extraction and purification of medicinal plants

Aug. 14, 2000, Bangkok, Thailand

Norio AIMI

Faculty of Pharmaceutical Sciences, Chiba University

Extraction and purification of medicinal plants

1) Purverization
2) Extraction

2-1) Extraction with solvents

Removal of fat or wax by n-hexane or pet. Ether

Extraction with appropriate solvents such as;

MeOH, EtOH, Acetone, AcOEt, Benzene,

Toluene, etc.

2-2) Supercritical fluid extraction

3) Separation and purification

3-1) Separation with a separatory funnel

Separation of ;

neutral and neutral substances

basic and neutral substances

acidic and neutral substances

3-2) Chromatography

Column chromatography, Liquid chromatography, Thin layer chromatography, Gas chromatography, Paper chromatography etc.

a) Ordinary phase chromatography

SiO_2, Al_2O_3 etc.

b) Reversed-phase chromatography

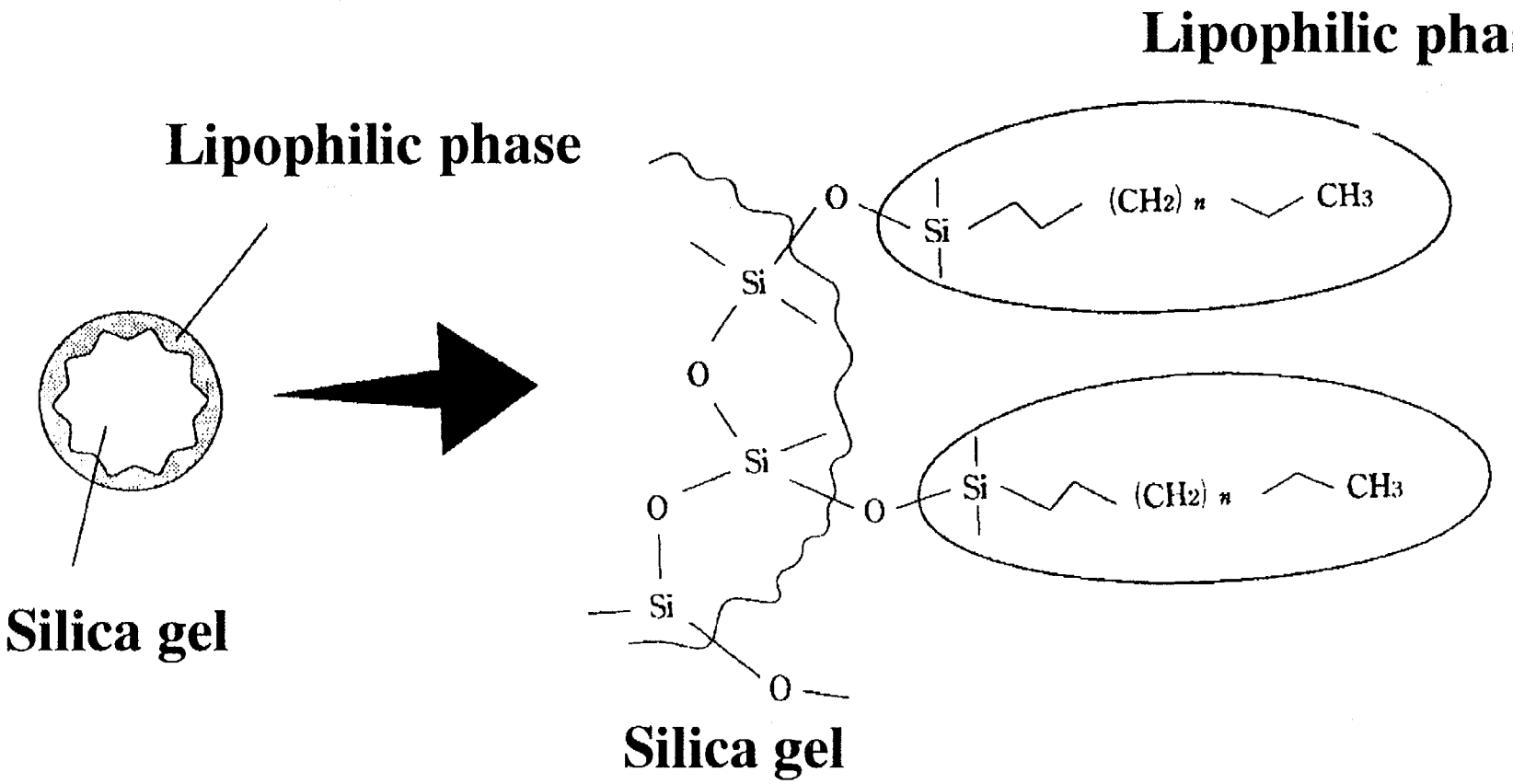
polyamide,

C_8, C_{18} (ODS) etc.

c) Gel permeation chromatography

porous polymer (Amberlite® XAD 4, Diaion® etc.)

d) Ion exchange chromatography



An example of the stationary phase of liquid chromatography

3-3) Crystallization

3-4) Distillation

4) Structure determination

4-1) Molecular formulae

Elemental analysis

High resolution mass spectrometry

ex)

CO;	**27.9949**
N_2;	**28.0062**
C_2H_4;	**28.0312**

4-2) Structure determination

4-2-1) Structure determination with chemical methods

4-2-2) Structure determination with spectroscopic methods

a) Mass spectrometry

Ionization;

EI (electron impact)

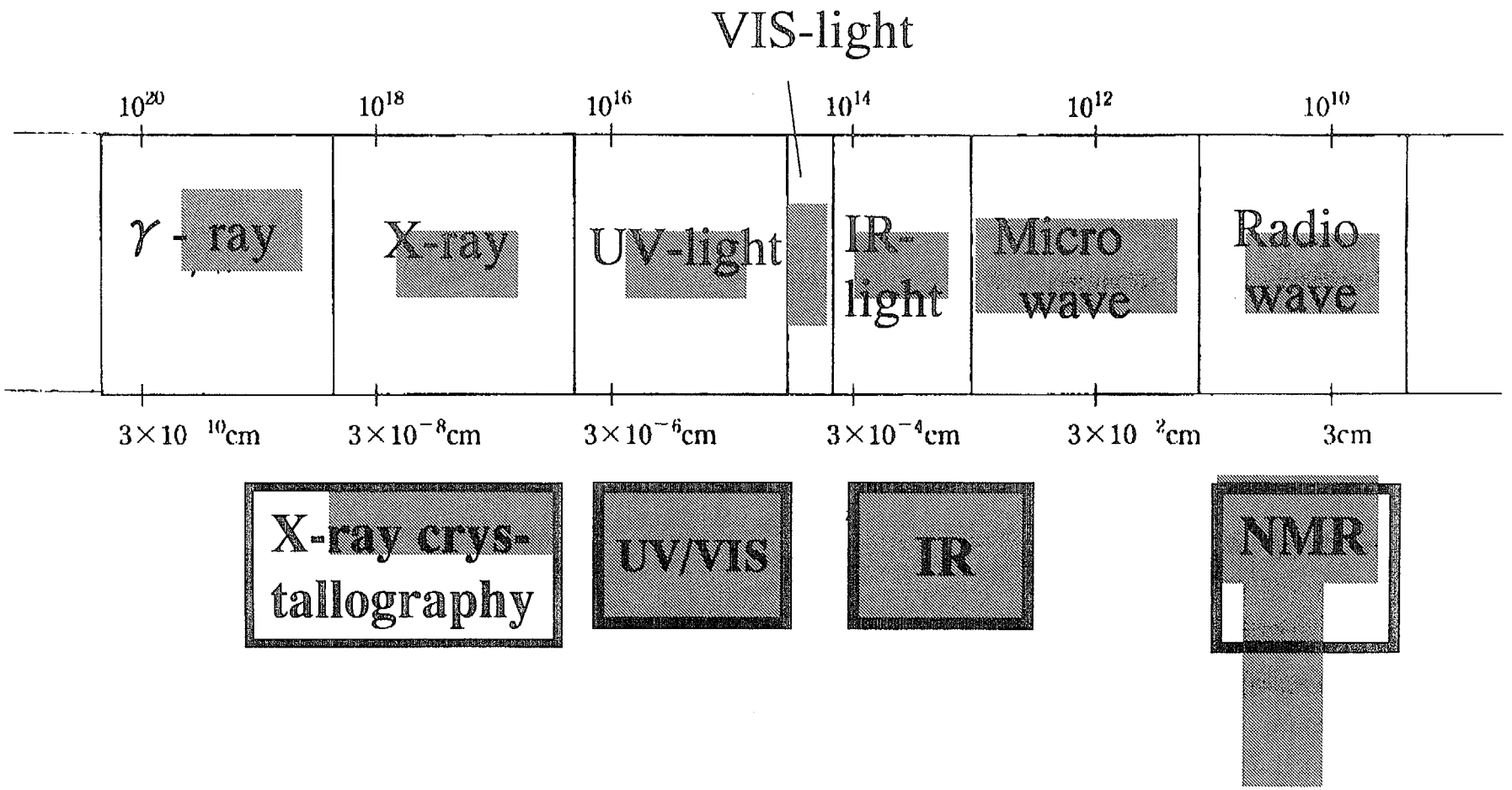
FAB (fast atom bombardment)

SIMS (secondary ion mass spectrometry)

FD (field desorption)

ESI (Electrospray Ionization) etc.

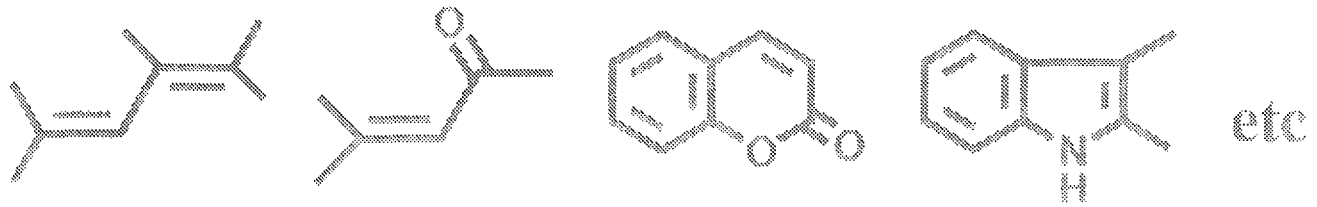
Organic spectroscopy



b) Spectroscopic methods

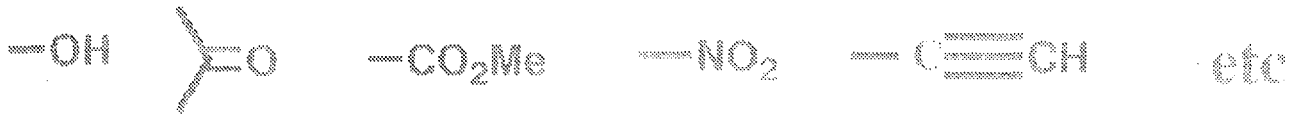
Ultraviolet-visible (UV) spectroscopy

gives information about Basic chromophore or conjugation of π electrons



Infrared spectroscopy (IR)

gives information about Functional groups



NMR spectroscopy (NMR)

gives information about **Structures around Carbon or Hydrogen**

Measurements

1D: ^{1}H-NMR, ^{13}C-NMR

Spin-spin decoupling measurement, NOE measurement, etc.

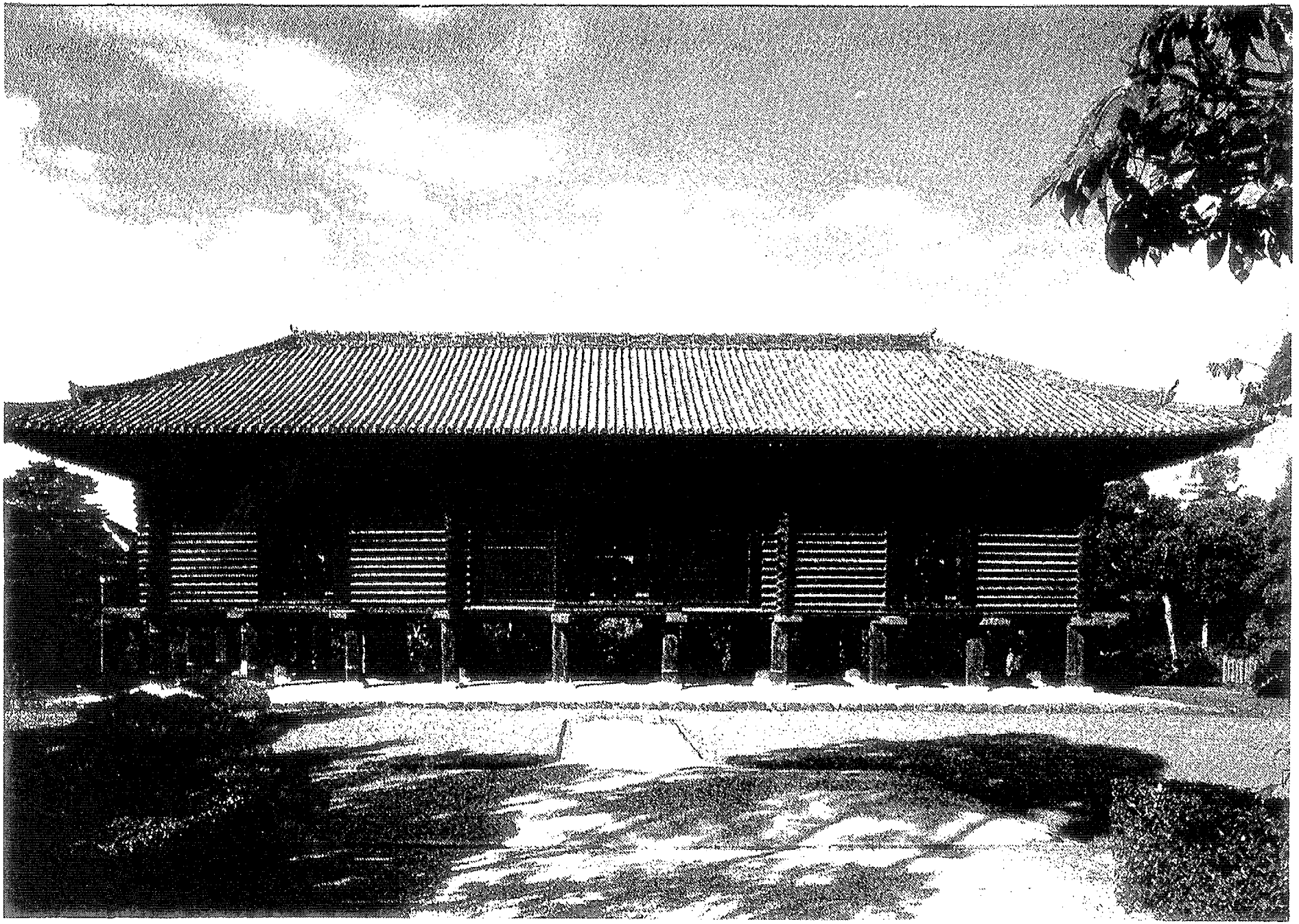
2D: ^{1}H-^{1}H COSY, NOESY, HOHAHA, DEPT, HMBC, etc.

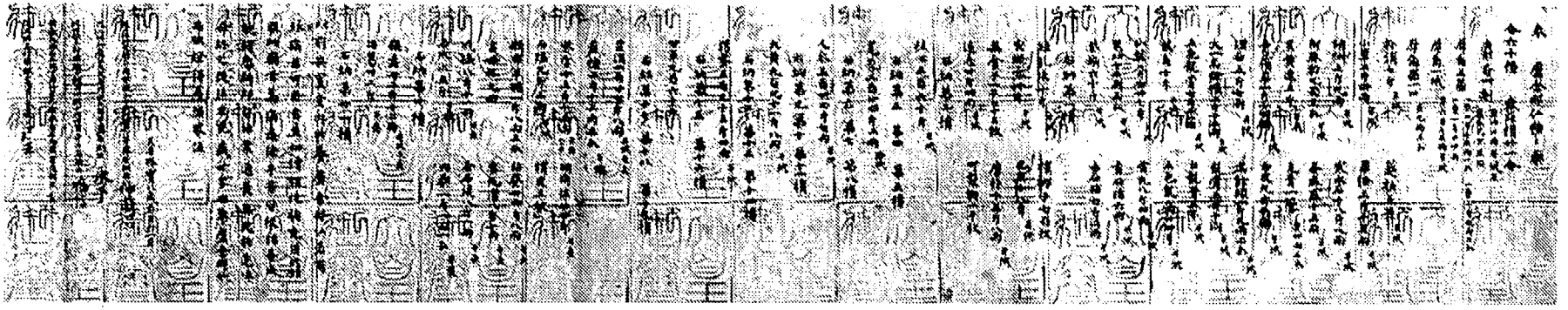
CD (Circular dichroism), ORD (Optical rotatory dispersion) spectroscopy

4-2-3) Structure determination with X-ray crystallography

Shôsôin Treasury

An azekura-type storehouse (33 meters long, 9.4 meters wide, and 14 meters high) with a floor 2.7 meters above the ground, was built at Nara in the eighth century. It contains over 10,000 articles of that century or earlier, possessions of Emperor Shômu that were presented to the Tôdaiji Temple by Empress Dowager Kômyô after Shômu's death in 756. The collection includes valuable works of fine art made in Japan and in various countries along Asia's silk road, as well as a wide variety of ancient tools, musical instruments and documents. Because storage conditions of the Shôsôin have been superb, and the Imperial court has made special efforts to preserve the building and its treasures, the Shôsôin and its collection are famous throughout the world for their unique cultural value.





Shuju-yaku-cho (種々薬帳): The dedicatory record of 60 herbal and mineral medicinal drugs contributed to the Big Buddah of Todaiji temple by the Empress Dowager Komyo (光明皇太后) on June 21, Tempyo-shoho-8th year (天平勝寶8歳、A. D. 756), on the occasion of seven -times-seventh day (七七忌) of the late Emperor Shomu (聖武天皇).

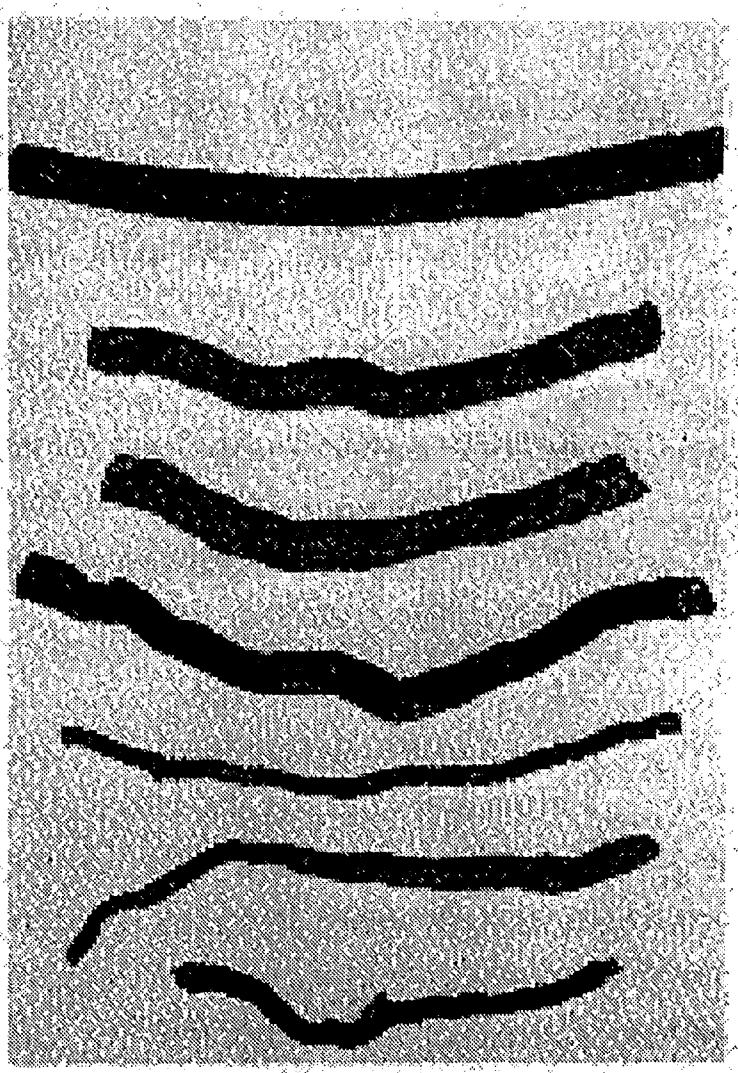


Fig. 2. N127 Uyaku-no-zoku (右楽127), (高麗2楽). Photograph offered from the Office of the Shosoin Treasure House, the Imperial Household Agency, Japan.

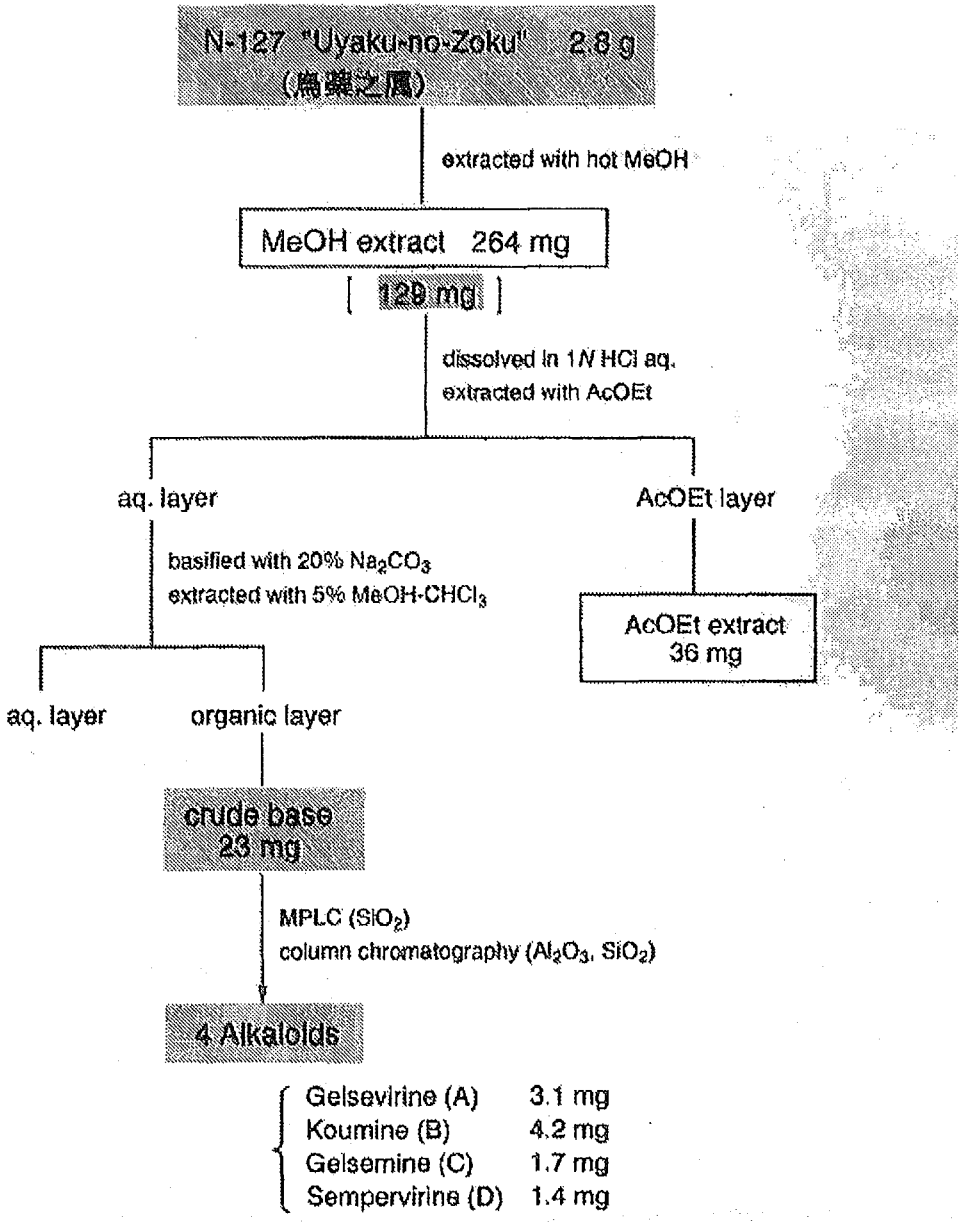
Second Scientific Investigation on Shosoin Medicinal

(One week each, October 1994 and October 1995)

Shoji Shibata, M. J. A., Emer. Prof., Univ. of Tokyo

Masao Konoshima, Emer. Prof., Kyoto Univ., (Deceased in March, 1996)
Mizuo Mizuno, President, Gifu College of Pharmacy
Tsuneo Namba, Prof., Toyama Med. & Pharm. Univ.
Norio Aimi, Prof., Chiba Univ.
Kaisuke Yoneda, Assoc. Prof., Osaka Univ.
Toru Okuyama, Prof., Meiji College of Pharmacy

Extraction and Separation of N-127 "Uyaku-no-Zoku"



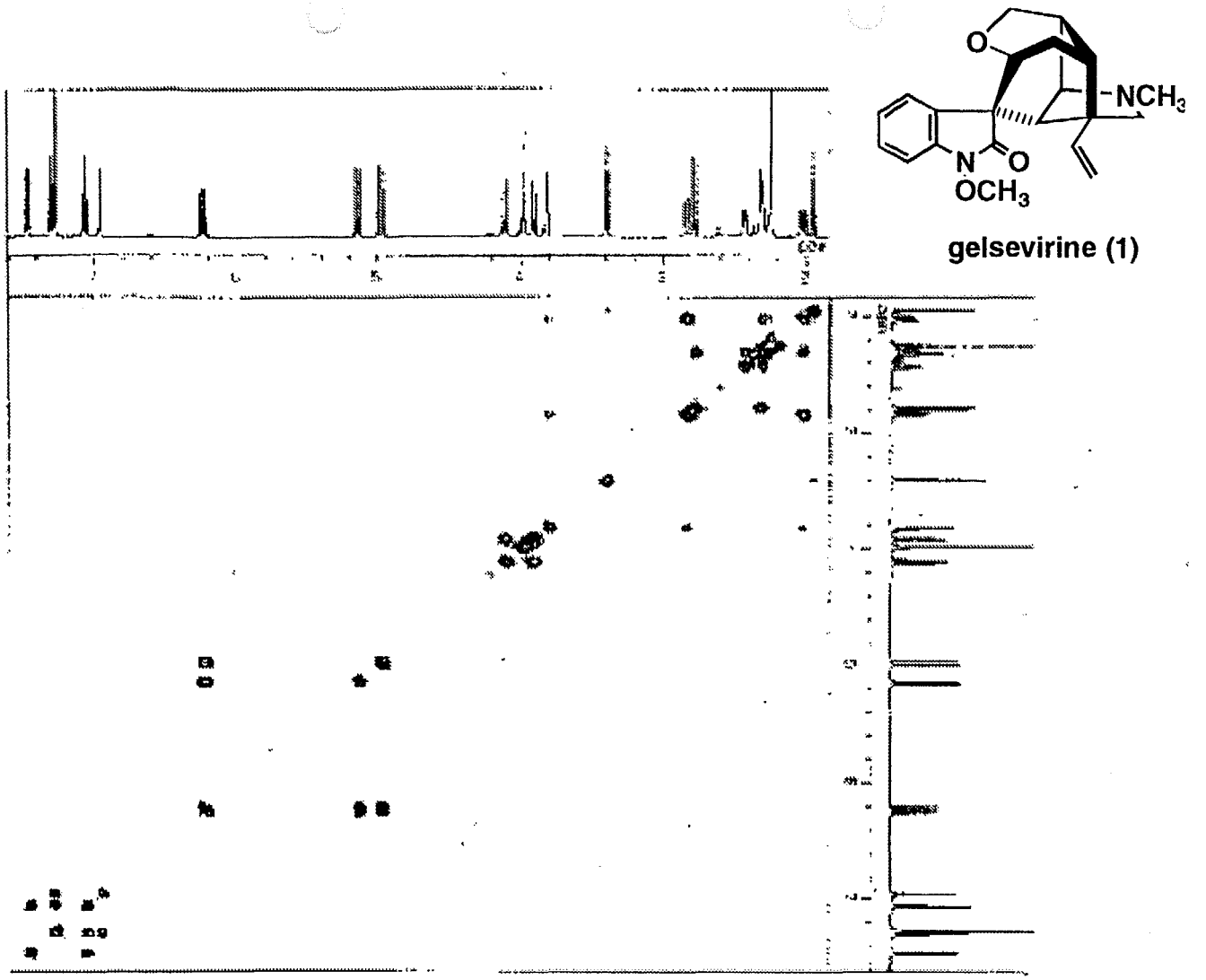
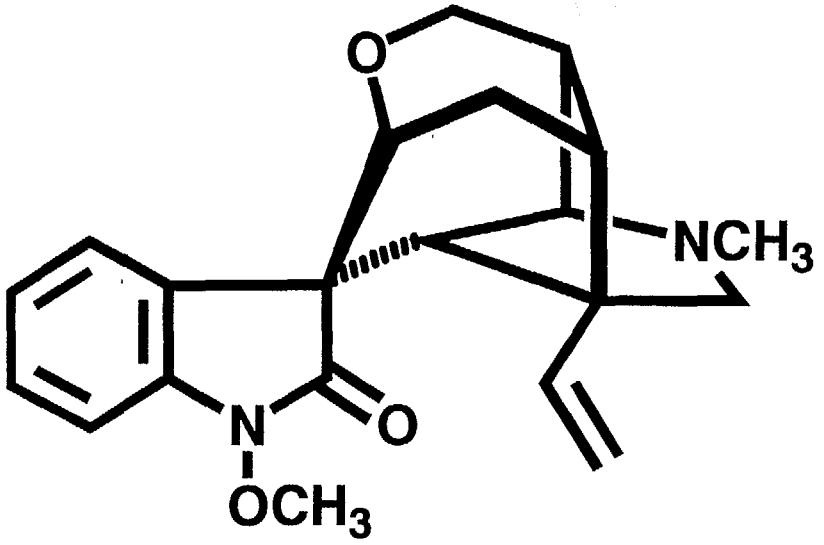
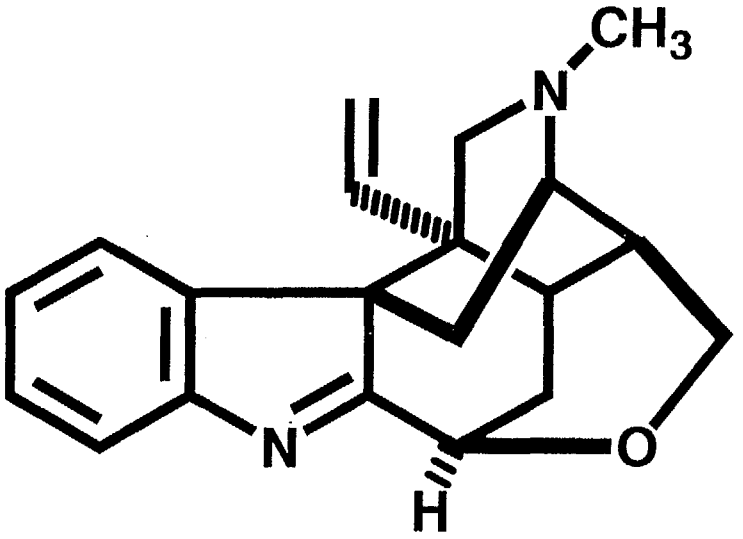


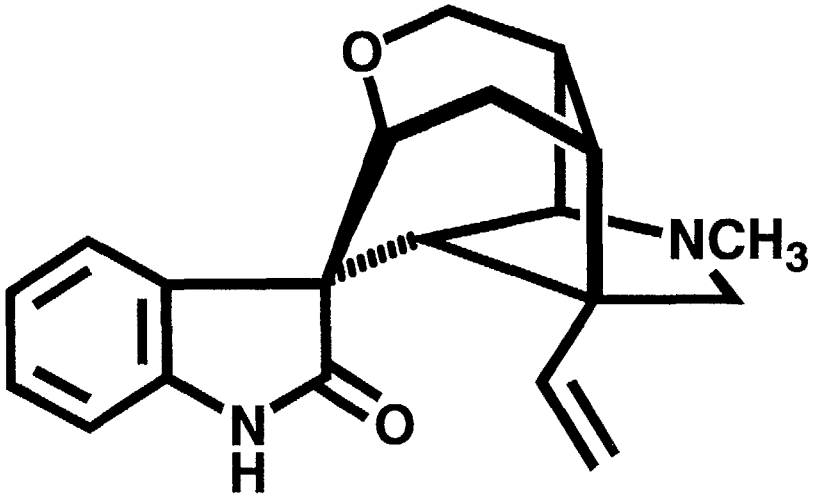
Fig. 1. A field gradient HH-COSY spectrum (500 MHz) of gersevirine (1) isolated from Yakatsu (冶佐) stored in Shosoin repository.



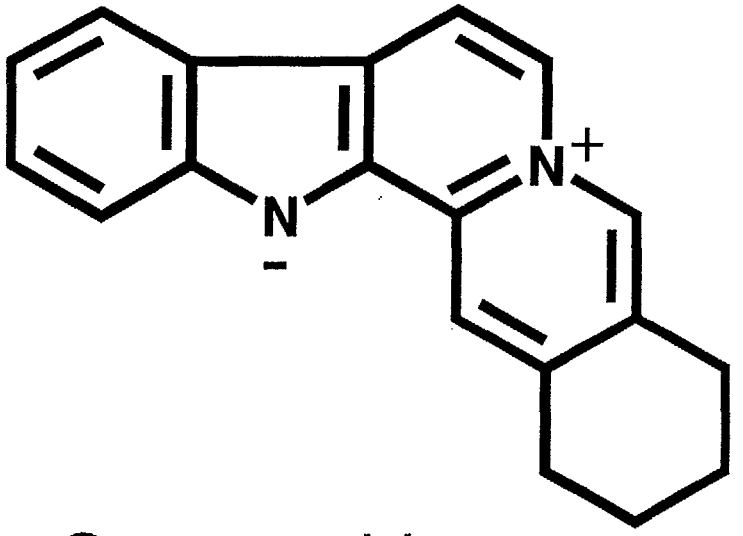
Gelsevirine



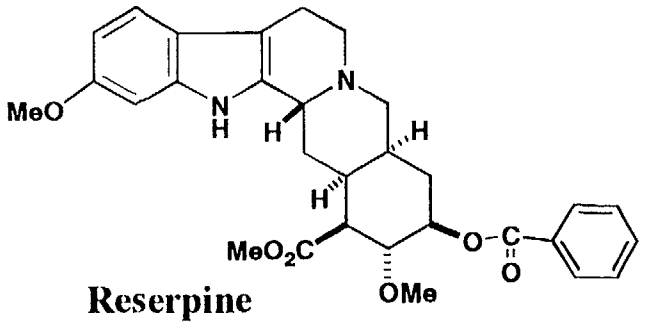
Koumine



Gelsemine

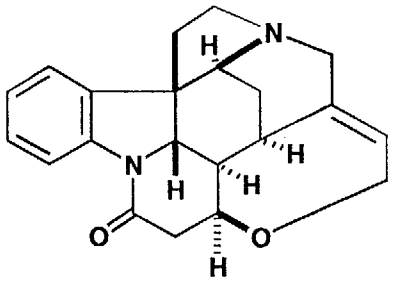


Sempervirine



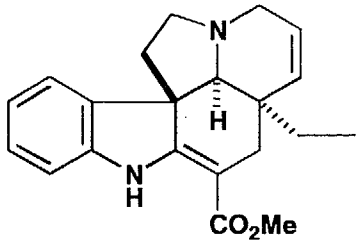
Reserpine

***Corynanthe*-type**



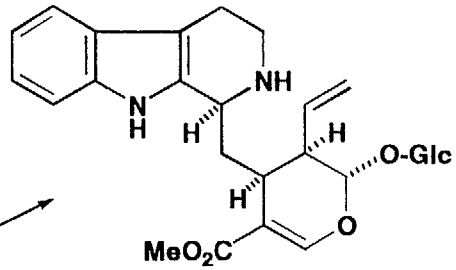
Strychnine

***Strychnos*-type**

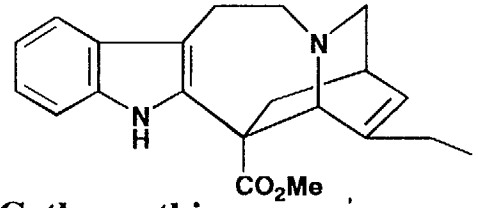


Tabersonine

***Aspidosperma*-type**

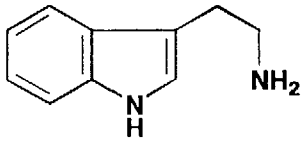


Strictosidine

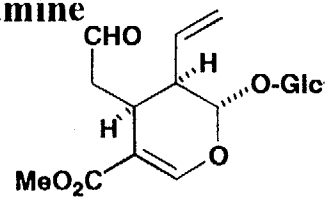


Catharanthine

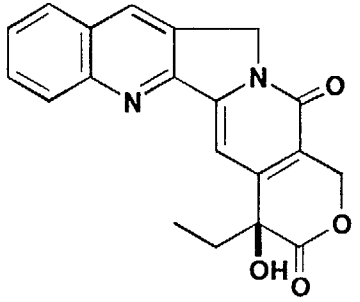
Iboga-type



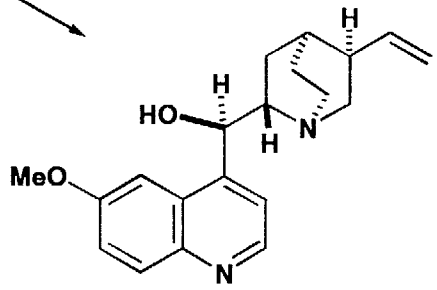
Tryptamine



Secologanin

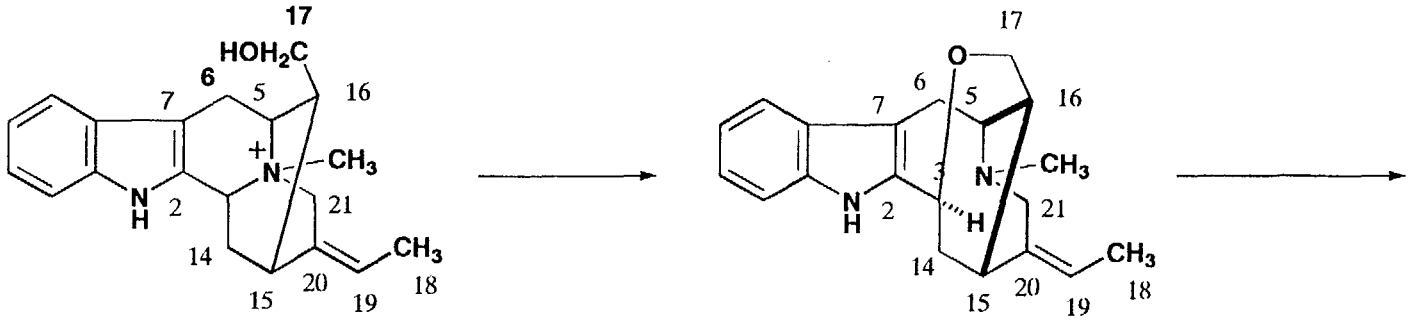


Camptothecin

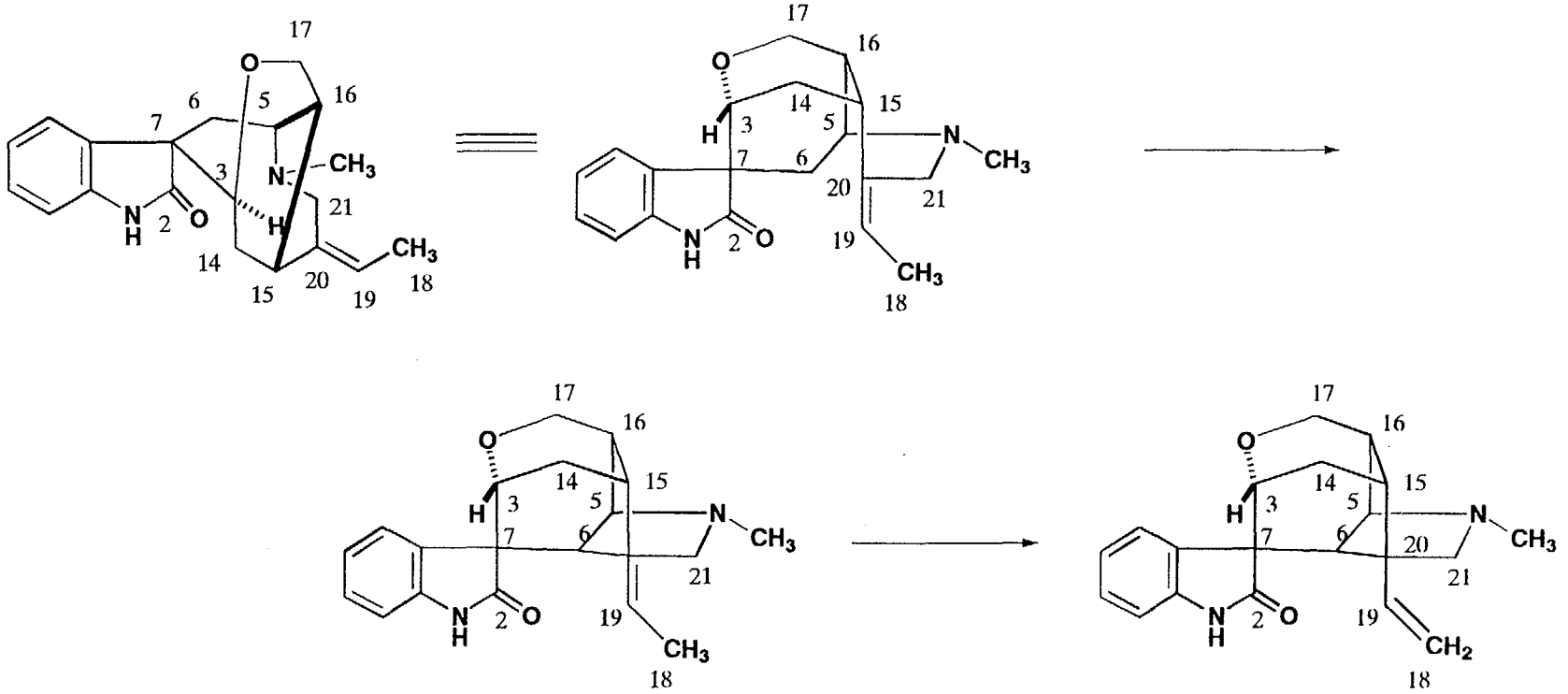


Quinine

***Cinchona* alkaloid**



Corynanthe / sarpagine-type alkaloid



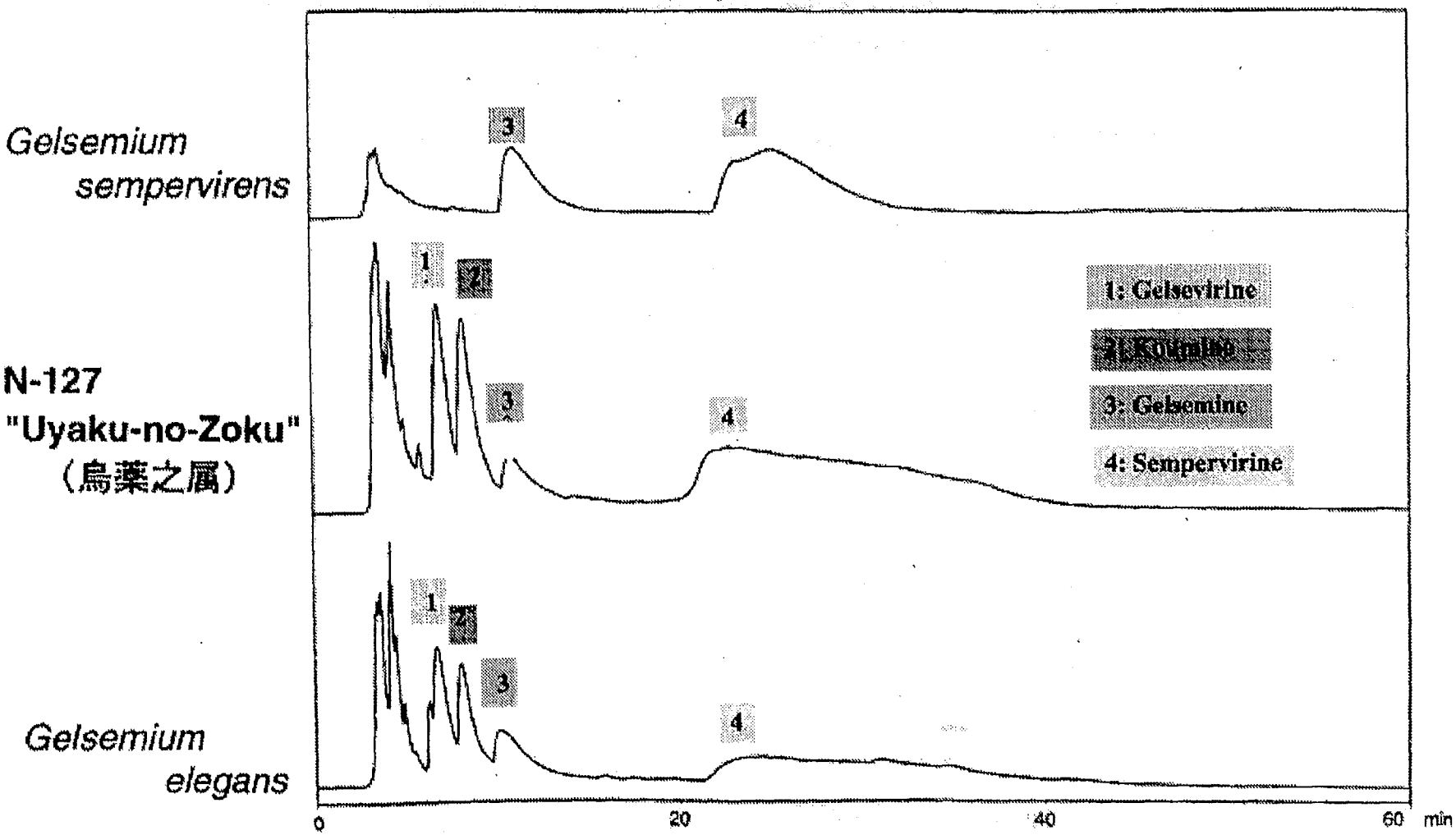
gelsemine

(Gelsemium Alkaloids)

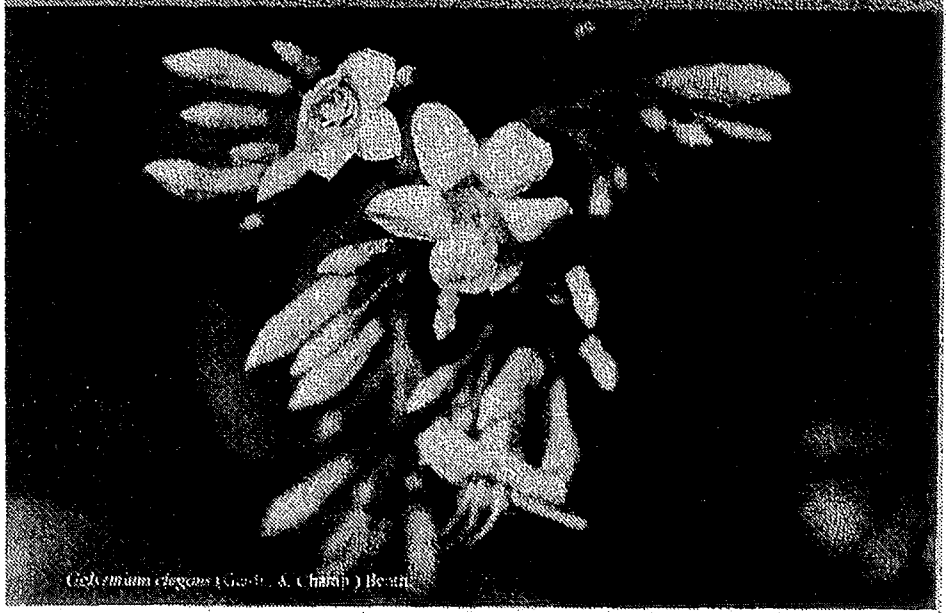
HPLC

column TSK-GEL SILICA-150 SI15C0042
solvent 10%MeOH-$CHCl_3$

flow rate 1.0ml/min
UV 254nm



FLORA OF THAILAND



Clerodendrum elegans (Graham) A. Chamb.) Benth.

VOLUME SIX

PART THREE

CRUCIFERAE, BEGONIACEAE, IXONANTHACEAE, LINACEAE, LOGANIACEAE, THYMELAEACEAE

THE FOREST HERBARIUM, ROYAL FOREST DEPARTMENT

BANGKOK 1992

Gardneria ovata (Griff. & Champ.) Benth. J. Linn. Soc. Bot. 1: 90. 1856; Kurz, Fl. Burm. 2: 249. 1877; Dop in Fl. Gén. I.-C. 4: 162. 1912; Kerr in Fl. Siam. En. 3: 51. 1951; Leenhouts in Fl. Mal. I. 6: 343. Fig. 27. 1962; Tirel-Roudet in Fl. C.L.V. 13: 69. 1972; Hô, Cây-cỏ Việtnam 2: 839. Fig. 6041. 1992.— *Medicia elegans* Gardn. & Champ. in Hook., J. Bot. Kew Misc. 1: 324. 1829. Figure 89. Plate XX: 2.

Stems much branched, terete. *Leaves*: petiole 1–1.5 cm; lamina ovate-lanceolate, 5–14 by 2–5 cm; apex long acuminate; bas rounded to decurrent, 6–12 secondary veins. *Inflorescence* compact, 3–12 cm, glabrous; bracteoles 1–2 mm, lanceolate, ciliate. *Flowers* with long slender pedicels to 6 mm. *Calyx* ca 0.5–1 mm, ciliate. *Corolla* ca 15 mm, yellow or orange; tube ca 7 mm, red-spotted inside; lobes reflexed, ca 7 mm, usually acute sometimes apiculate or rounded. *Stamens* exserted beyond the corolla tube, filaments ca 1 cm, attached at base of corolla tube; anthers 2 mm, oblong. *Ovary* with 1 style, together ca 8–12 mm. *Fruit* ca 1.5 cm, with 8–10 seeds per cell. *Seeds* flattened, ca 4 mm, winged, often strongly laciniate, centre minutely hairy.

Thailand.— NORTHERN: Chiang Mai, Phrae; SOUTH-WESTERN: Kanchanaburi; PENINSULAR: Krabi, Narathiwat.

Distribution.— India, Sri Lanka, Laos, Vietnam, Malay Peninsula, Sabah, Sarawak, China (type Hong Kong), Sumatra, Java.

Ecology.— Forests, sometimes on limestone, alt. 600–1500 m.

Vernacular.— Kok muan (กกม่วน) (Udon Thani), ma khet (มะเข็ด), sam bai tai (สามใบยาย), mali narok (มะลินรก) (Nan).

Uses.— Whole plant, but especially leaves and roots, very poisonous (various alkaloids), traditional uses can be used as fish poison and in general control against various parasites. Used extensively for sporting and suicide (a single cup-of-tea made with three leaves from the plant is lethal).

Note.— Thai material appears totally glabrous except for some marginal cilia, whereas from elsewhere in SE Asia it may be sparsely pubescent (especially in the inflorescence).

3. GARDNERIA

Wall. in Roxb. Fl. Ind. 1: 400. 1820.

Glabrous woody climbers. *Leaves* petiolate. *Stipules* forming a small rim. *Flowers* small, 4–5-merous, borne in dichasia, occasionally solitary. *Calyx* small, deeply lobed. *Corolla* valvate in bud. *Stamens* exserted. *Fruit* a globular berry. *Seeds* patelliform.

About 5 species in South-east Asia; 1 species in Thailand.

Gardneria ovata Wall. in Roxb., Fl. Ind. 1: 400. 1820; Kurz, Fl. Burm. 2: 227. 1877; Kerr in Fl. Siam. En. 3: 63. 1951; Leenhouts in Fl. Mal. I. 6: 361. Fig. 33. 1972.

Stem much branched, terete. *Leaves*: petiole 1–1.5 cm; lamina lanceolate to ovate, 3–13 by 1.5–3.5 cm; apex acute; base decurrent, 4–10 secondary veins. *Inflorescence*

Acknowledgements

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Dr. Hiromitsu Takayama, Assoc. Prof. , Faculty of Pharm. Sci., Chiba Univ.

Dr. Mariko Kitajima, Research Associate, Faculty of Pharm. Sci., Chiba Univ.

Dr. Dhavadee Ponglux, Assoc. Prof. , Faculty of Pharm. Sci., Chulalongkorn Univ. Thailand

Dr. Sumphan Wongseripipatana, Assoc. Prof. , Fac. of Pharm. Sci., Chulal. Univ. Thailand

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Dr. Kensuke Sakurai, Former Researcher, Shionogi Research Institute

Ms. Rei Uematsu, Essayist

CHEMICAL MODIFICATION OF NATURAL PRODUCTS FOR MODERN DRUG DEVELOPMENT

Professor Norio Aimi

Chemical modification of natural products for modern drug development

Aug. 14, 2000, Bangkok, Thailand

Norio AIMI

Faculty of Pharmaceutical Sciences, Chiba University

Camptothecin Story

1950 – 1959

Large scale screening program for cortisone precursors was made by M. E. Wall at the Eastern Regional Research Laboratory, USDA, Philadelphia, PA. Plant collection was conducted by botanists under the authpices of the Plant Introduction Division of the USDA.

USAD: United States Department of Agriculture

Discovery of camptothecin

Biodirected isolation in the L1210 mouse leu-kemia life prolongation assay.

"In general, after conducting a particular phase of the isolation, it would often take us 3 months or more before the results were received. This was primarily due to the fact that the L1210 assay is based on life prolongation. In the case of highly active extracts the study would have to be carried out for 30 days or more. Although it was a slow process, we persisted, working at the same time on other plant bioassay-directed fractionations, one of which resulted in the isolation of taxol."

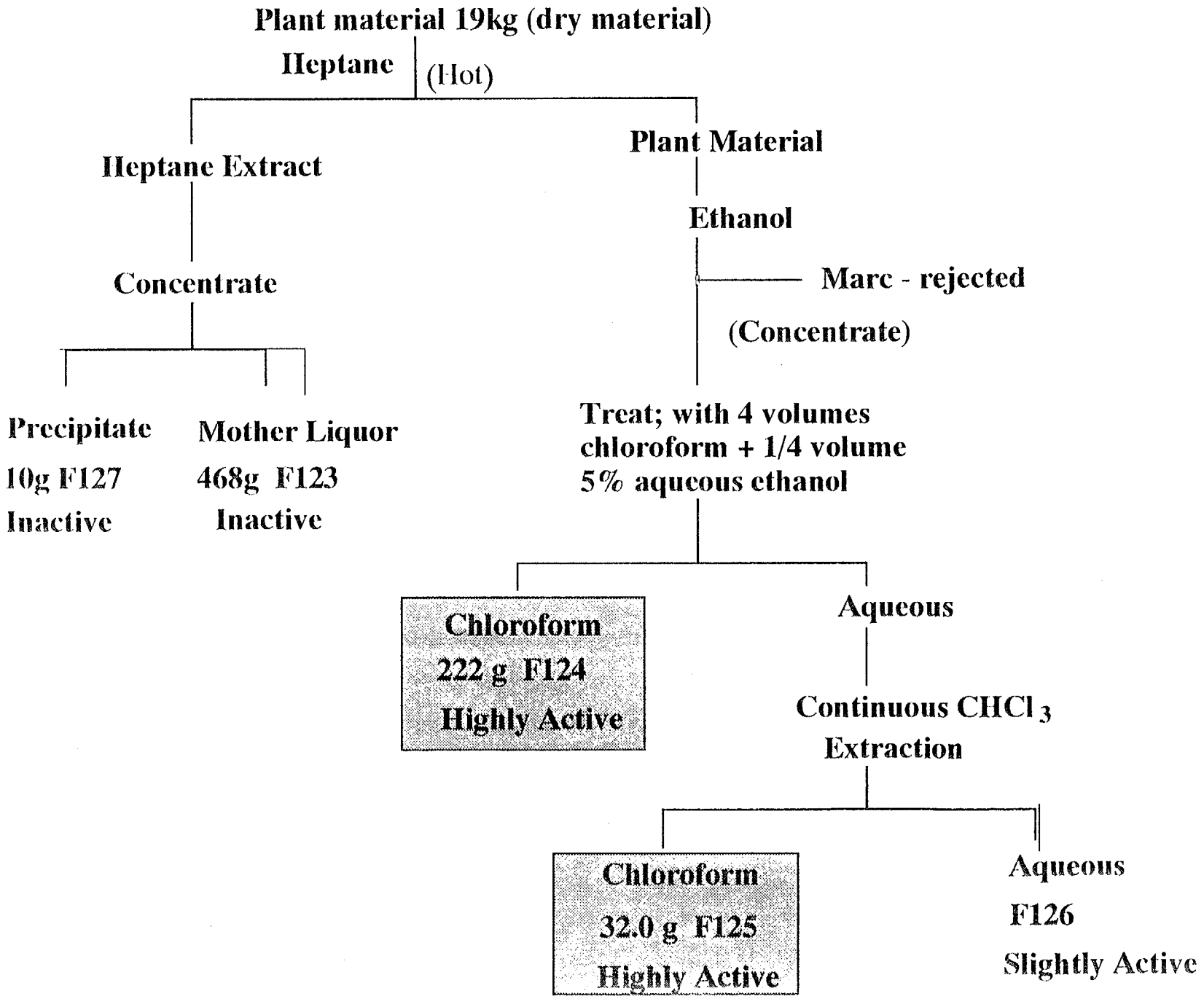
M. E. Wall and M. C. Wani, *Cancer Research*, 55, 753-60 (1995)

Camptotheca acuminata Decne

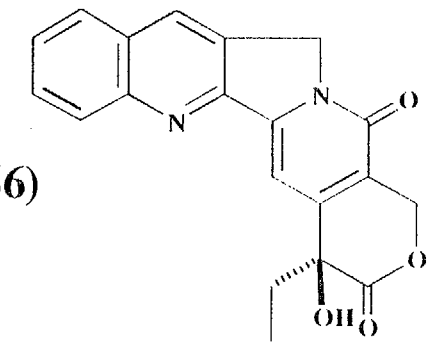
Family: Nyssaceae （ヌマミズキ科）

Chinese Name: 喜樹

Distribution: Southern provinces of China; Szechwan, Yunnan, Kwangsi


Plant material 19kg (dry material)
Heptane
(Hot)
Heptane Extract
Plant Material
Concentrate
Ethanol
Marc - rejected
(Concentrate)
Precipitate
10g F127
Inactive
Mother Liquor
468g F123
Inactive
Treat; with 4 volumes
chloroform + 1/4 volume
5% aqueous ethanol
Chloroform
222 g F124
Highly Active
Aqueous
Continuous CHCl 3
Extraction
Chloroform
32.0 g F125
Highly Active
Aqueous
F126
Slightly Active

Physical properties of Camptothecin



M. E. Wall et al., *J. Am. Chem. Soc.*, 85, 3888 - 3890 (1966)

Yellow needles, mp 264-267 (decomp)

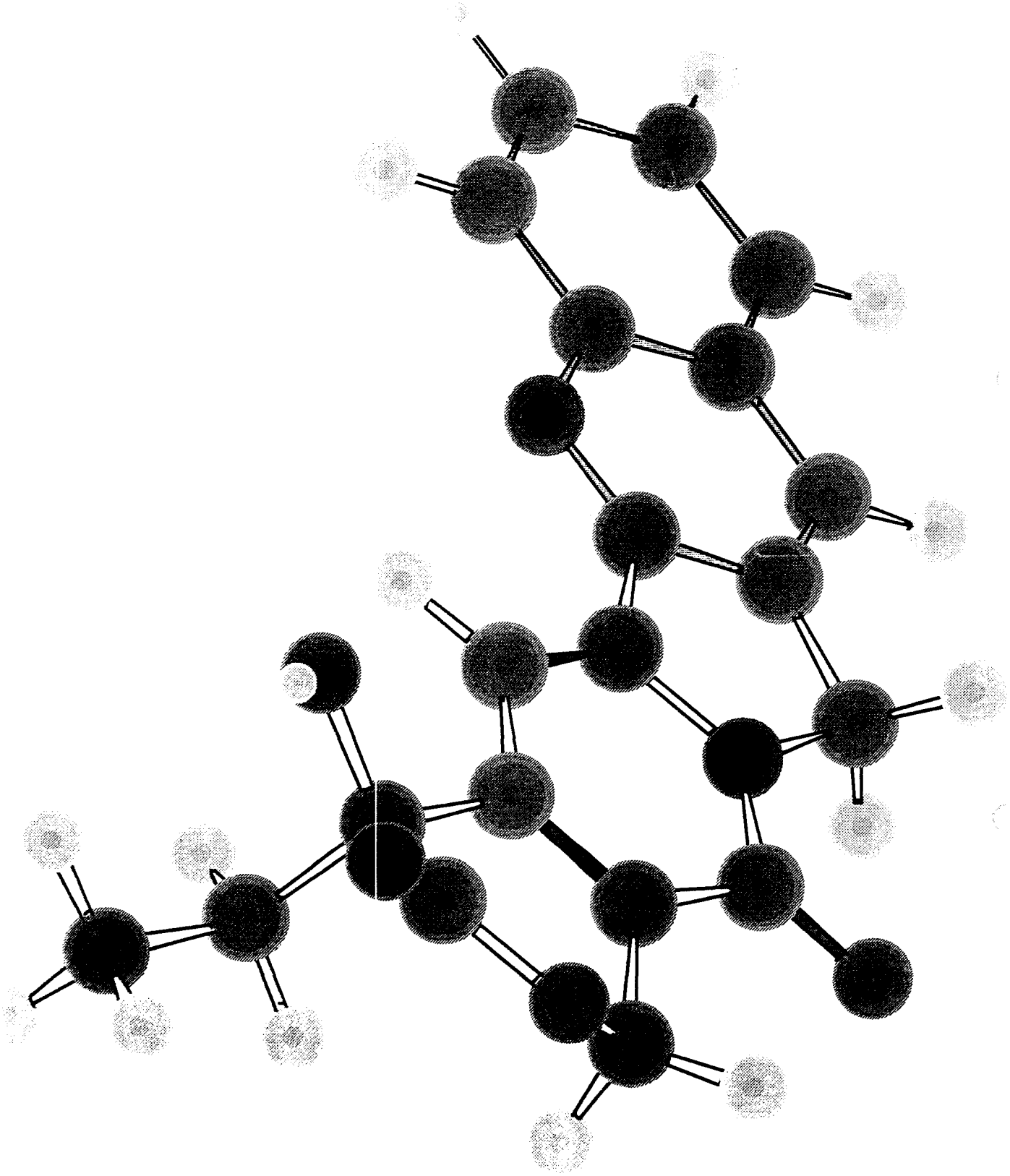
$C_{20}H_{16}N_2O_4$ Found: m/z 348.1117; Calcd.: m/z 348.1111

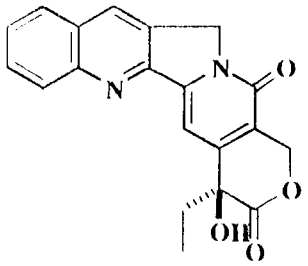
$[\alpha]_D$ +31.3

λ_{max}nm: 220 (ε 37,320), 254 (29,230), 290 (4,980), and 370 (19,900)

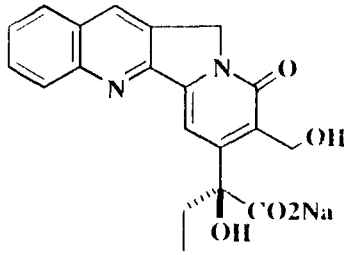
$\nu_{max}cm^{-1}$: 3440 (OH), 1760 - 1745 (lactone), 1660 (lactam), and 1610, 1585 (arom. C=C)

δ ppm (60MHz, CD_3SOCD_3):0.91 (3H, t, -$CH_2C\underline{H_3}$), 1.90 (2H, m, C(OH)-$C\underline{H_2}CH_3$), 5.45 (2H, Ar-$C\underline{H_2}$O-), and 5.28 (2H, Ar-$C\underline{H_2}$-N)

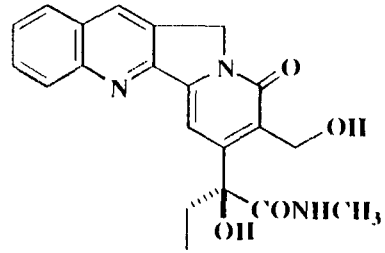




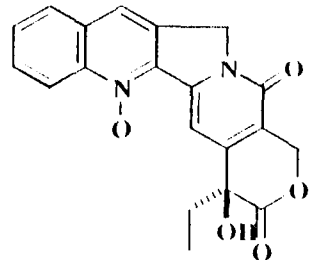
Camptothecin
L1210, T/C 220 at 2 mg/kg
PS, T/C 197 at 4 mg/kg



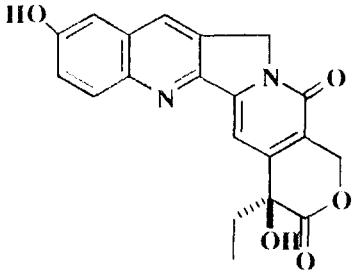
Camptothecin sodium
L1210, T/C 209 at 3 mg/kg
PS, T/C 212 at 40 mg/kg



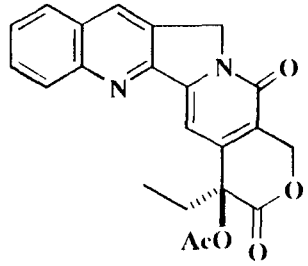
L1210, T/C 172 at 3.5 mg/kg



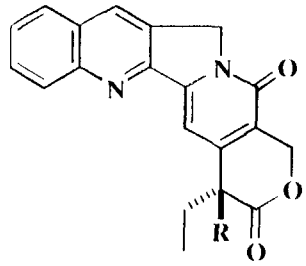
L1210, T/C 144 at 2 mg/kg



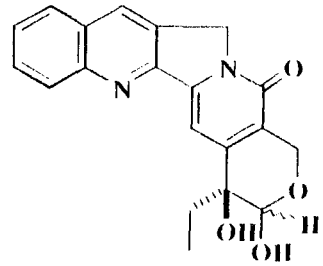
10-Hydroxy camptothecin
L1210, T/C 230 at 0.5 mg/kg
PS, T/C 314 at 4 mg/kg



Slightly active
L1210, T/C 125 at 25 mg/kg



R = Cl
R = H
Inactive



Inactive

Comparison of in vivo and in vitro activity of CPT and its analogs

CPT derivative	Inhibition of relaxation of super-coiled DNA (%)	DNA scission (%)		Antitumor activity[b] (I/C) × 100		ED_{50} (mg/ml)	
		Supercoiled[a]	Linear[a]	L1210	P388	9KB[c]	9PS
S	52	48	41	197 (8)	197 (4)	10^{-2}	10^{-2}
R	20	12	9	<125	–	10^{-1}	10^{-0}
R,S	30	25	15	–	222 (8)	10^{-1}	–
20-deoxy-(*R,S*)	0	0	1	<125	–	–	–
10-OH-(*S*)	73	71	40	348 (20)	297 (3)	10^{-2}	–
11-OH-(*R,S*)	74	35	31	357 (60)	–	–	
10-OCH^3-(*R,S*)	–	–	–	167 (2)	–	10^{-2}	
10,11-$(CH_2O)_2$-(*R,S*)	0	0	2	<125	<125	10^{-1}	
10,11-OCH^2O-(*R,S*)	62	82	40	325 (2)	–	10^{-2}	
10-NO_2-(*R,S*)	19	23	22	219 (16)	–	10^{-1}	
11-NO_2-(*R,S*)	0	11	11	147 (80)	–	10^{-0}	
12-NO_2-(*S*)	0	10	3	151 (40)	151 (40)	–	
9-NO_2-(*S*)	40	50	22	348 (10)	–	10^{-2}	
9-NH_2-(*S*)	91	35	31	348 (2.5)	–	10^{-2}	
10-NH_2-(*R,S*)	16	16	18	325 (8)	–	10^{2}	
11-NH_2-(*R,S*)	33	7	14	147 (40)	–	10^{-1}	
12-NH_2-(*S*)	0	0	3	<125	<125	–	
21-Lactam-(*S*)	3	0	2	178 (40)	–	10^{-1}	
Tricyclic-(*R,S*)	0	0	–	<125	–	–	

[a]Effect of 10 mM of test compound as a percentage of the effect of the control; cf. Jaxel et al. (1989).

[b]Mouse leukemias L1210 and P388, inoculated intraperitoneally; drug administered intraperitoneally on days 1 and 5. T/C is survival time of treated/control animals × 100. The dose (mg/kg) giving the greatest reported T/C value is given in parentheses. Antitumor data are from Wani et al. (1986, 1987a).

[c]Inhibition of cell growth in 9KB expressed as ED_{50} (mg/ml).

THE TOTAL SYNTHESIS OF *dl*-CAMPTOTHECIN

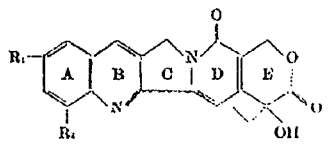
Shanghai No. 5 Pharmaceutical Plant (上海第五制药厂),
Shanghai No. 12 Pharmaceutical Plant (上海第十二制药厂),
Shanghai Institute of Pharmaceutical Industrial Research
(上海医药工业研究院),
and
Shanghai Institute of Materia Medica (上海药物研究所)*

Received October 16, 1976.

ABSTRACT

Recently, a brief account of the total synthesis of *dl*-camptothecin through 10-step reactions starting from 3-cyano-4-methyl-6-carbethoxy-2(1H)-pyridone in 18% overall yield was reported[1]. In this article, the detailed procedures of the synthesis are presented.

Camptotheca acuminata, belonging to the family Nyssaceae, is an indigenous plant distributed widely and abundantly in the southern provinces of China. An antitumour alkaloid camptothecin (I) was isolated from the stem and root of this plant by Wall and his associates, and the chemical structure of this alkaloid has subsequently been elucidated to be a pentacyclic compound containing the functioning moieties of a quinoline, a lactam and a lactone ring[2]. The novel structural features as well as its marked antitumour activity have since then inspired many organic chemists world wide with interest in synthesizing this alkaloid. So far a number of successful total syntheses have been reported[3]. Recently, in Shanghai Institute of Materia Medica this alkaloid was isolated from the seeds of this plant[4], and after clinical trials it was found that camptothecin did possess certain therapeutic effect against gastric, rectum, colonic, and bladder tumours, but it seemed to be rather toxic. However, the reports that some camptothecin ring-A analogues, such as 10-hydroxy camptothecin (II) and 12-chloro-camptothecin (III)[5], did exhibit lower toxicity and broader antitumour spectrum in animals than the parent compound. As a result of this finding, the authors were encouraged to work out a more practical and general method for the total synthesis of camptothecin through which a series of ring A substituted analogues may easily be obtained. Here, in this report, a method for the total synthesis of *dl*-camptothecin is described. In this named total synthesis, there are ten working steps involved, starting from 3-cyano-4-methyl-6-carbethoxy-2(1H)-pyridone (IV) in 18% overall yield.

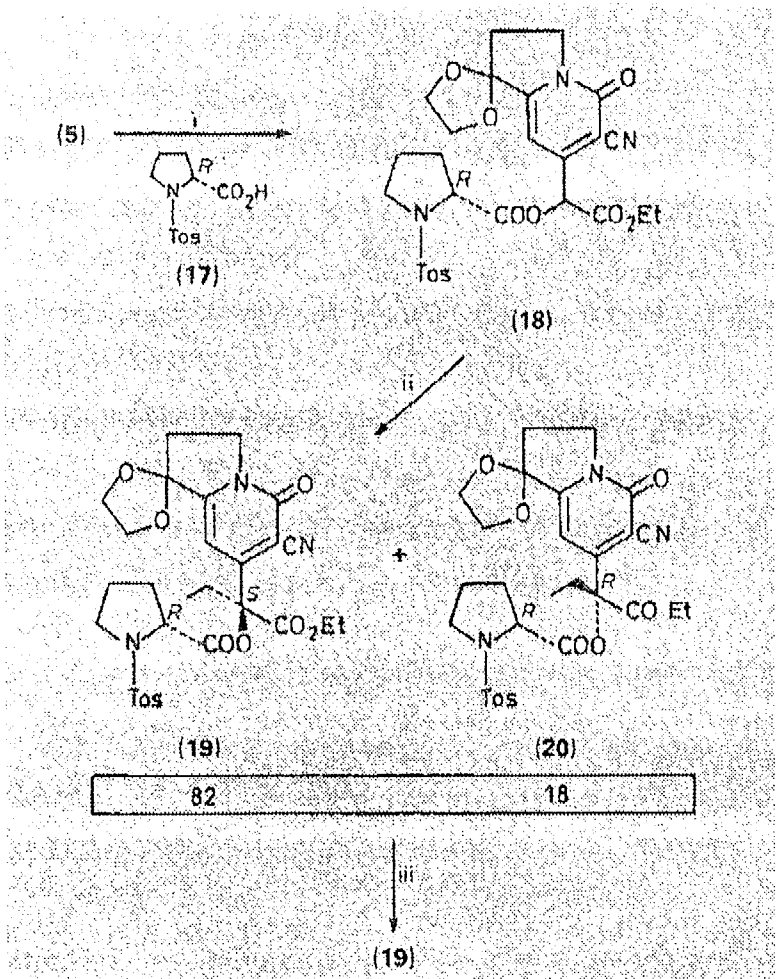


I. $R_1 = H$, $R_2 = H$
II. $R_1 = OH$, $R_2 = H$
III. $R_1 = H$, $R_2 = Cl$

* Correspondence should be addressed to this institute.

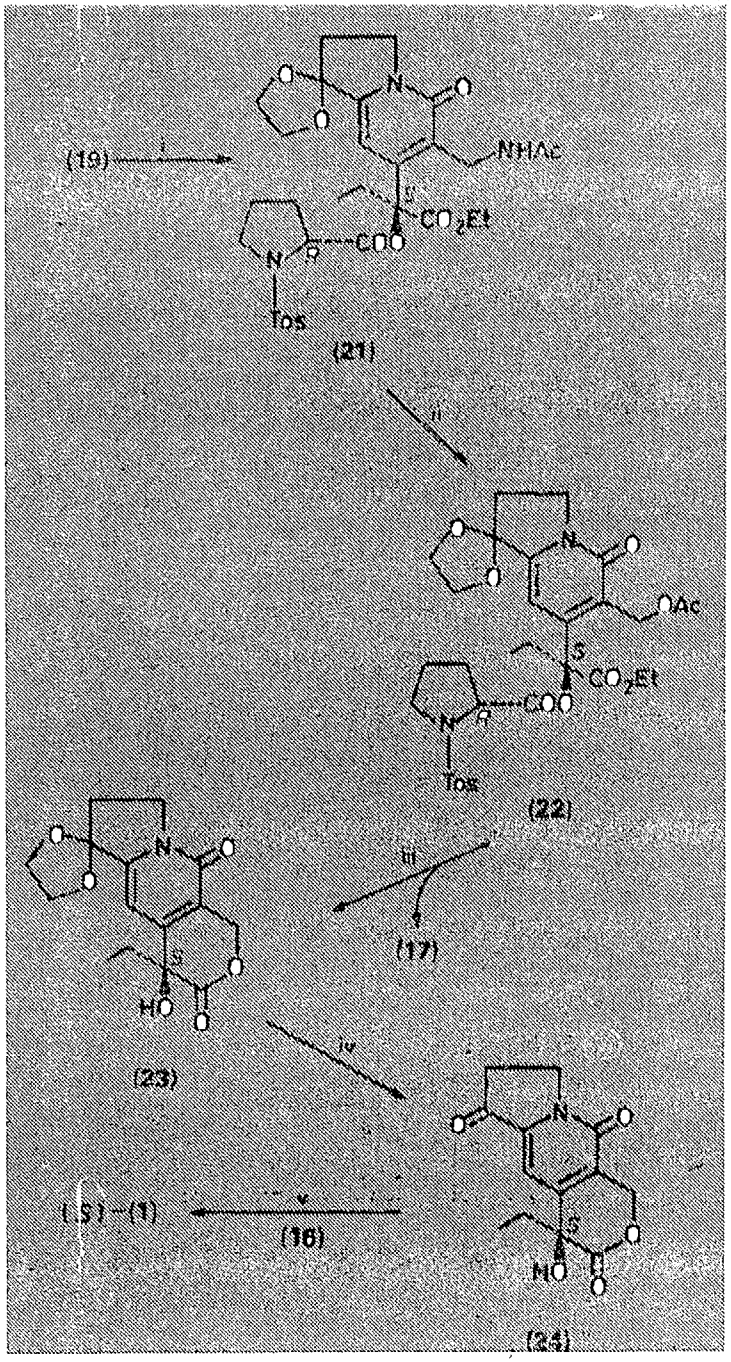
OH
O
N
O
O
O
NH2
CHO

OH
O
N
O
O
N

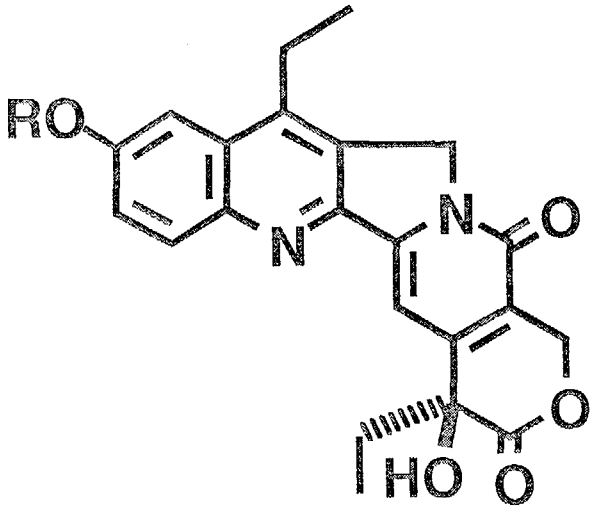


Scheme 3. *Reagents and conditions*: i, Na_2CO_3, DMF; ii, NaH, EtI, DMF; iii, recrystallization from propan-2-ol

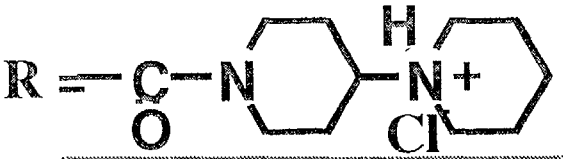
tetrahydroindolizin-7-yl]acetate (**6**).—To a solution of (**4**) (0.61 g, 2 mmol) in DME (12 ml) was added bromine (0.35 g, 2.2 mmol) and the resulting mixture was stirred for 6 h at room temperature. After a similar work-up procedure to that used for the preparation of (**5**) above, compound (**6**) (0.18 g, 23.5%) was obtained as a white solid, m.p. 147—151 °C (Found: C, 47.35; H, 3.9; N, 7.35. $C_{15}H_{15}BrN_2O_5$ requires C, 47.0; H, 3.95; N, 7.3%); R_F (A) 0.29; $\nu_{max.}$(KBr) 2 210, 1 725, and 1 650 cm^{-1}; $\delta(CDCl_3)$



Clinical medicines

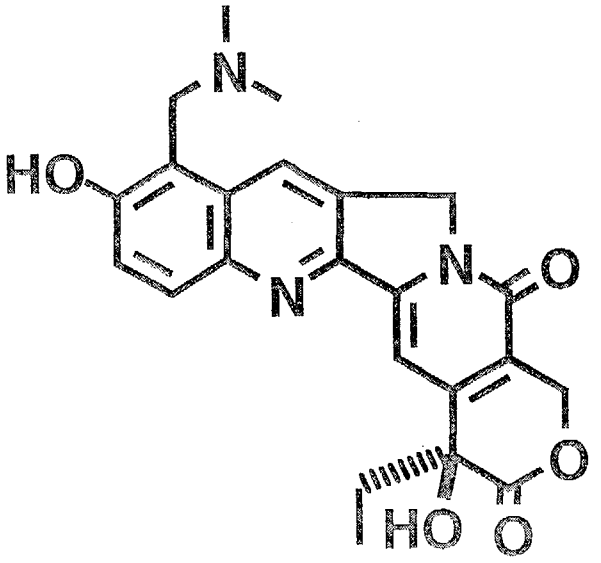


R = H: SN38



塩酸イリノテカン (CPT11)

(ヤクルト、第一製薬)



Topothecan

(SmithKleine Beecham)

Camptothecin Producing Plants

Camptotheca acuminata Decne; Nyssaceae

M. W. Wall, M. C. Wani, C. E. cook, K. H. Paler, A. T. McPhail, G. A. Sim, *J. Am. Chem. Soc.*, 88, 3888 - 3890 (1966)

Nothapodytes foetida (Wight) Sleumer; Icacinaceae (*Mappia foetida* Miers; Olacaceae)

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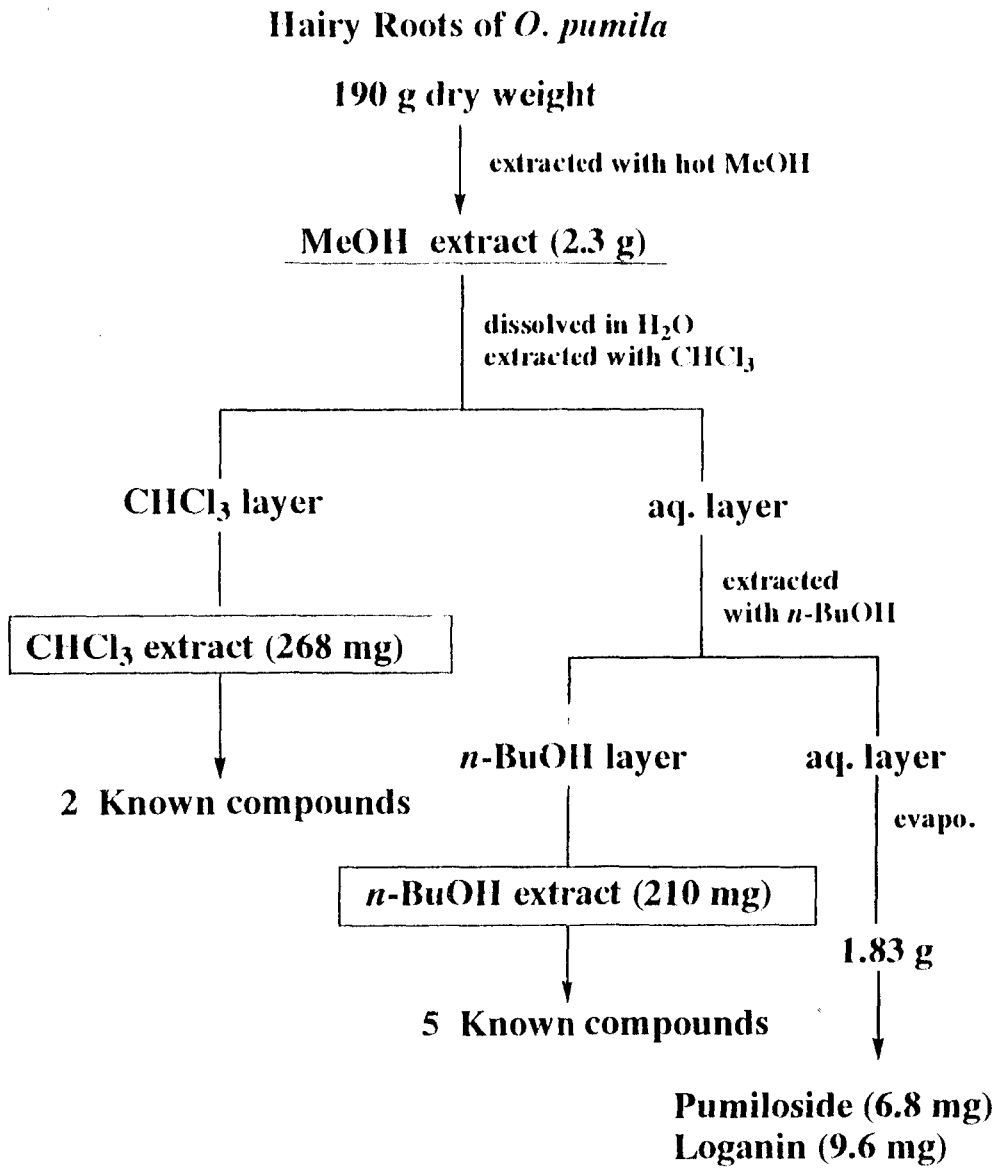
M. Arisawa, S. P. Gunasekera, G. A. Cordell, and N. R. Farnsworth, *Planta Medica*, 43, 404 - 415 (1981)

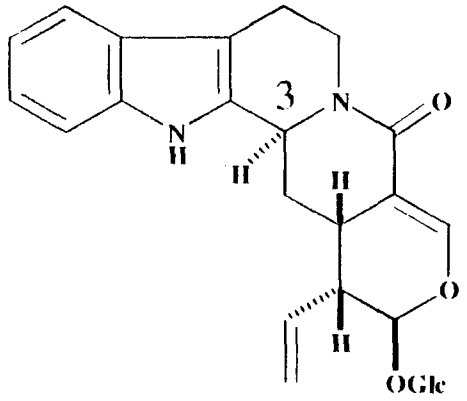
Ophiorrhiza Alkaloids

Finding of New Camptothecinoids

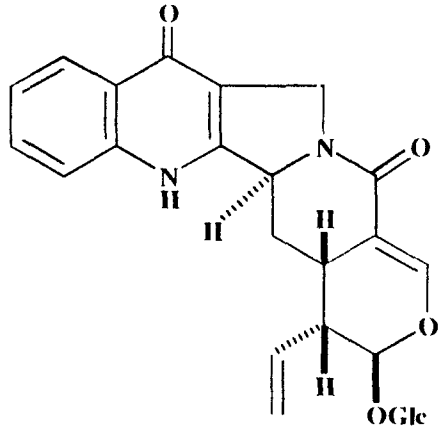
Mechanism of Biological Formation of Camptothecin

Production of Alkaloids by Cultured Cells of *Ophiorrhiza* spp.

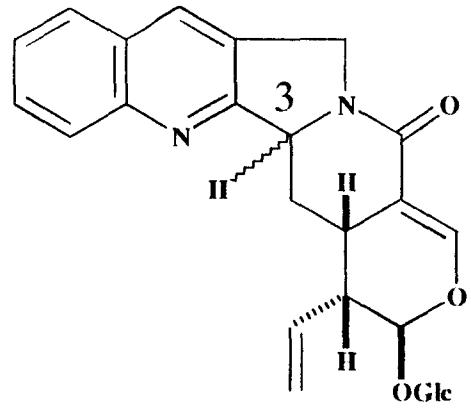

Hairy Roots of O. pumila
190 g dry weight
extracted with hot MeOH
MeOH extract (2.3 g)
dissolved in H2O
extracted with CHCl3
CHCl3 layer
aq. layer
extracted
with n-BuOH
CHCl3 extract (268 mg)
2 Known compounds
n-BuOH layer
aq. layer
evapo.
n-BuOH extract (210 mg)
1.83 g
5 Known compounds
Pumiloside (6.8 mg)
Loganin (9.6 mg)



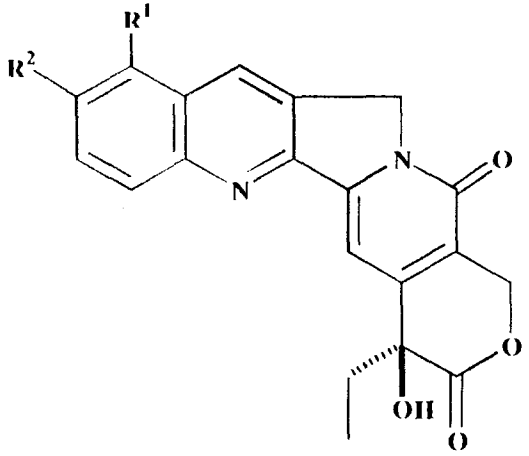
Strictosamide (2)



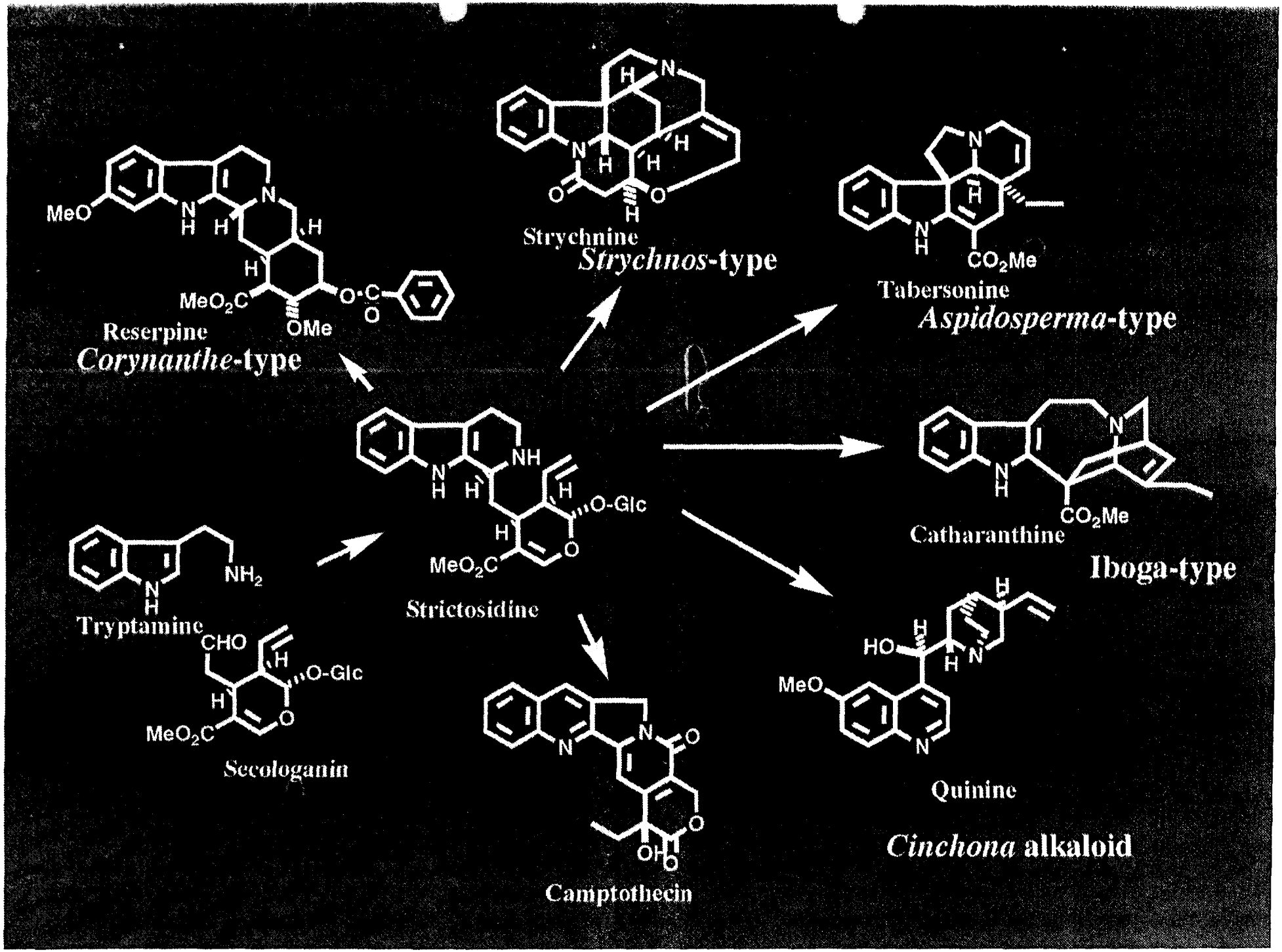
Pumiloside (3)

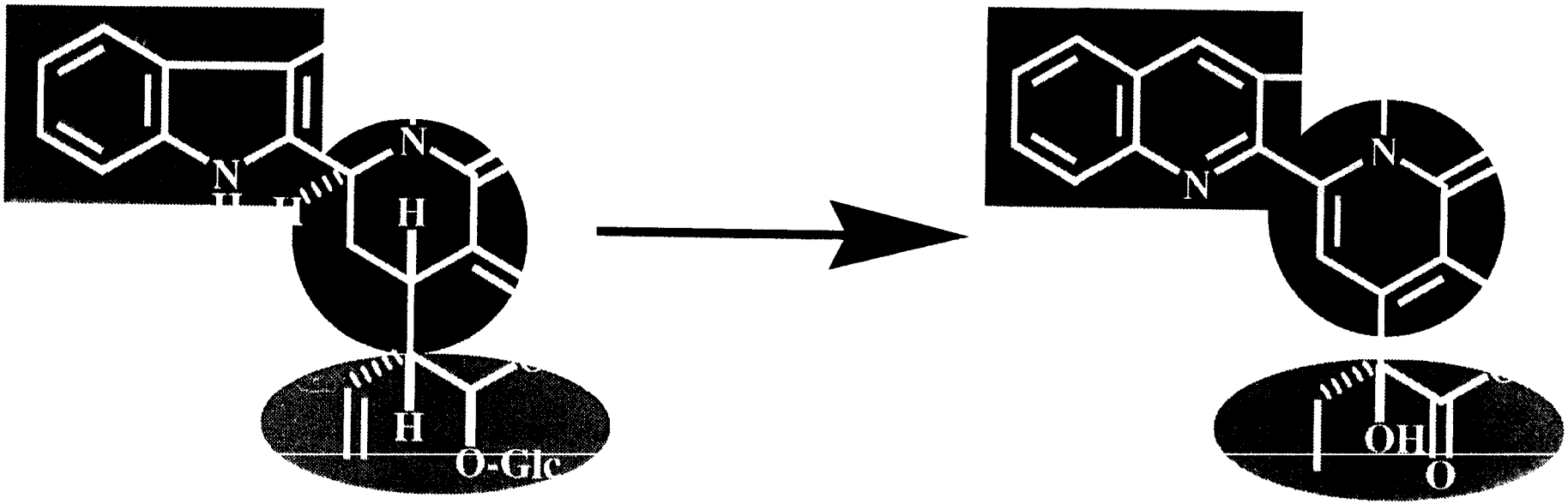


3α-H: 3 (*S*)-Deoxypumiloside (4)
3β-H: 3 (*R*)-Deoxypumiloside (5)

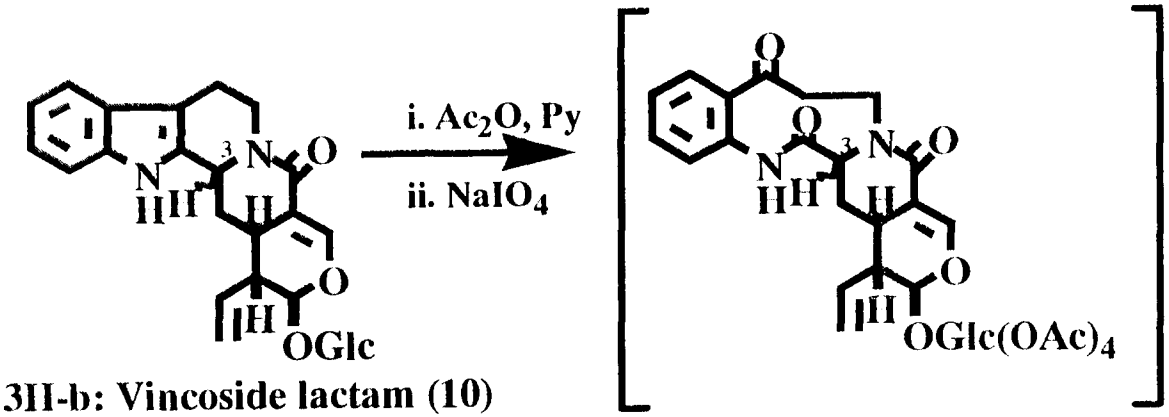


R^1=R^2=H: Camptothecin (1)
R^1=OMe, R^2=H: 9-Methoxycamptothecin (6)
R^1=OMe, R^2=OGlc: Chaboside (7)


Reserpine
Corynanthe-type
Strychnine
Strychnos-type
Tabersonine
Aspidosperma-type
Catharanthine
Iboga-type
Tryptamine
Strictosidine
Secologanin
Camptothecin
Quinine
Cinchona alkaloid

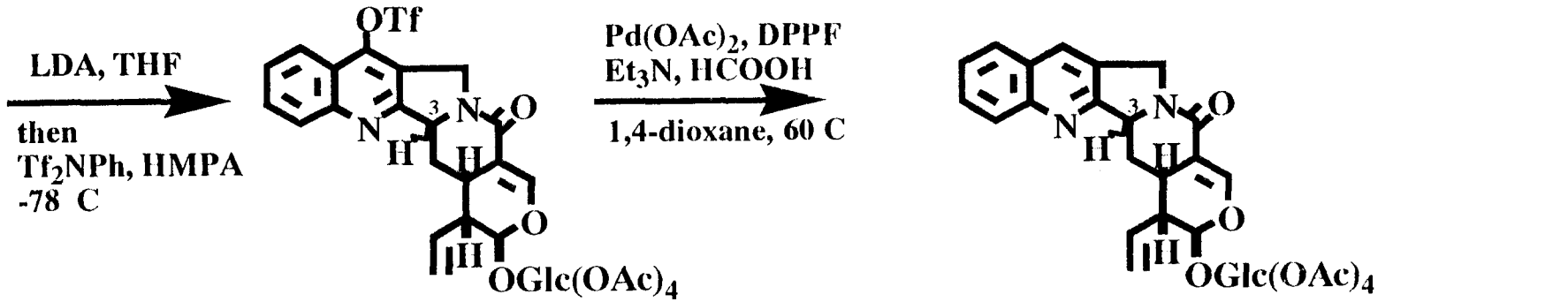


Strictosamide **Camptothecin**

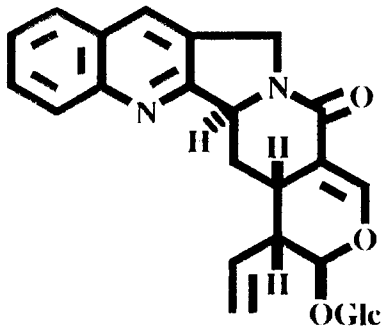


3II-b: Vincoside lactam (10)
3II-a: Strictosamide (2)

3II-β: (11)
3II-α: Pumiloside tetraacetate (13)



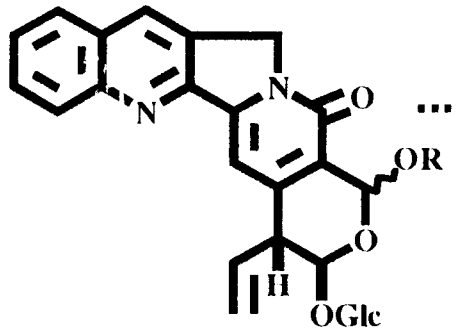
3H-β: 3(*R*)-Deoxypumiloside tetraacetate (12)
3H-α: 3(*S*)-Deoxypumiloside tetraacetate (14)



3(*S*)-Deoxypumiloside

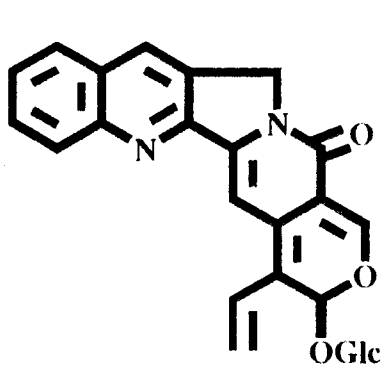
Oxydation

?



Compound X

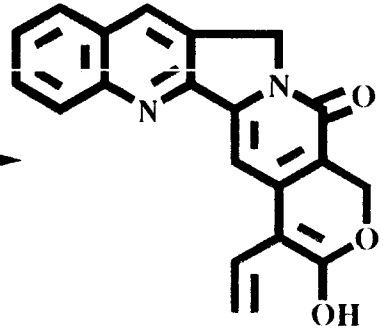
?



1) Hydrolysis

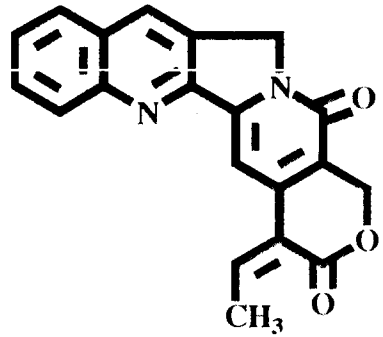
2) [1,5]-H-Transfer

?

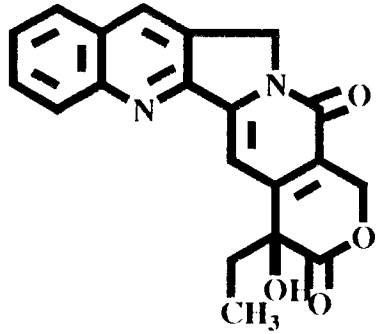


[1,5]-H-Transfer

?



?



Camptothecin

PRACTICAL TRAINING

* EXTRACTION & PURIFICATION OF MEDICINAL PLANTS

* CHEMICAL MODIFICATION OF NATURAL PRODUCTS

TINOSPORAE CAULIS

Tinospora Stem

GENERAL DESCRIPTION OF THE PLANT

Botanical name

Tinospora crispa **(L.) Miers ex Hook F & Th**

(Fam. **Menispermaceae**)

National name

Brunei :
Indonesia : Brotowali
Malaysia : Putarwali
Philippines : Makabuhay
Singapore : Akar Putarwali
Thailand : Boraphet

Scientific synonyms

Cocculus crispus **(L.) DC.** [2, 6]
G. Verrucosus **(Roxb. ex Fleming) Wall**
Menispermum crispum **L.**
M. tuberculatum **Lam.**
M. verrucosum **Roxb. ex Fleming**
Tinospora gibbericaulis **Hand. – Mazz.**
T. mastersii **Diels**
T. rumphii **Boerl**
T. thorelii **Gagnep**
T. tuberculata **(Lam.) Beumee ex. K. Heyne**

Morphological description

Woody climber, entirely glabrous. Young stems smooth, old stems prominently tuberculate and exceedingly bitter sap; aerial roots filiform, very long. Leaves broadly ovate to orbicular, 5-14 cm long and 4-12 cm wide; apex cuminate; base cordate; palmately 5-7 nerved at the base; petioles 5-15 cm long. Inflorescences often arising from the older and leafless stems. Male inflorescences very slender, pseudoracemose, flowers small, on filiform pedicels; sepals pale green, 3 outer ovate, 3 inner obovate; petals 3; stamens 6. Female inflorescences similar to male one but shorter. Female flowers with sepals and petals as in male; staminodes 6 ; carpels 3. Drupe orange, ellipsoidal, up to 2 cm long [2, 6]

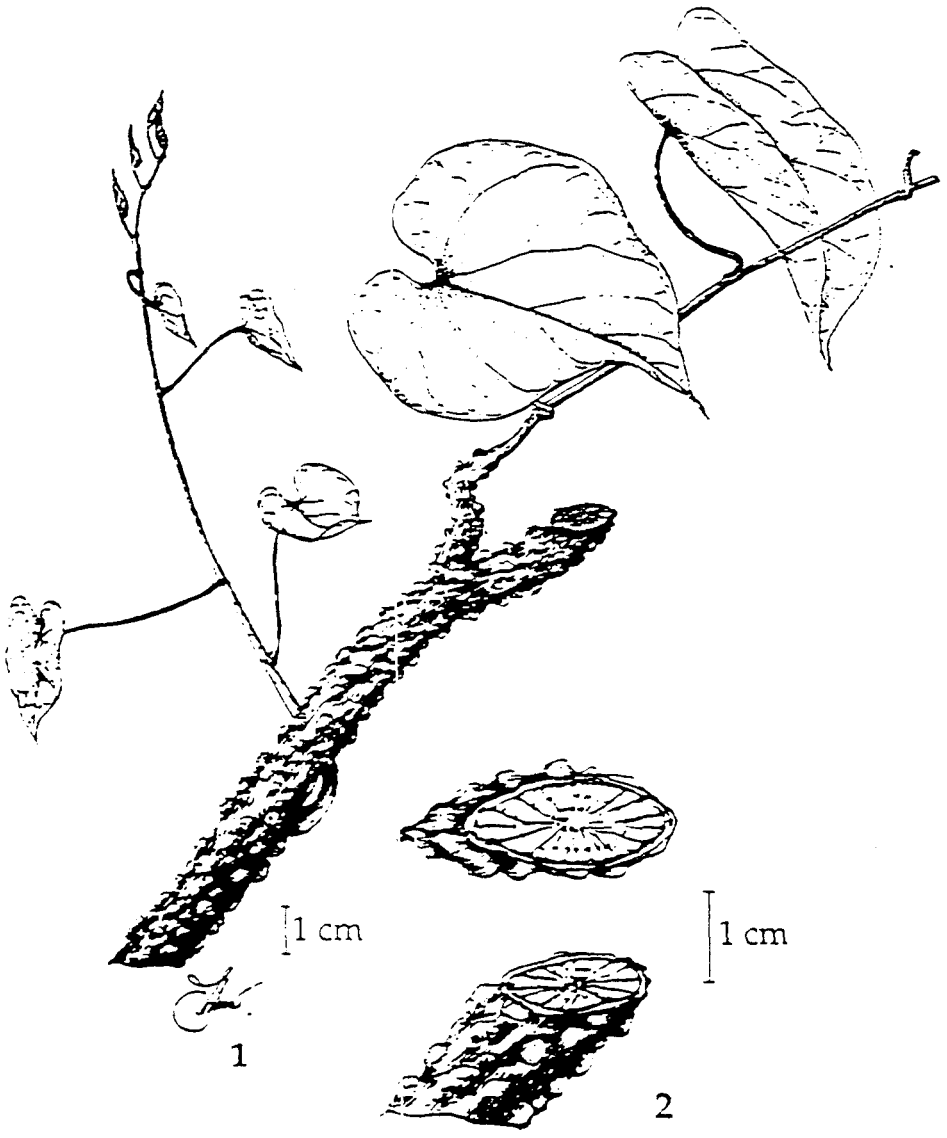


Fig. 1 *Tinospora crispa* (Linn.) Miers ex Hook. f. et Thoms.

1. branching stem
2. dried crude drug

Geographical distribution and local abundance

Brunei :

Indonesia : Grows wild or cultivated throughout tropical areas in Indonesia.

Malaysia :

Philippines : Throughout the country

Singapore :

Thailand : It is widely distributed throughout the country.

Habitat

This plant is commonly in the tropical Asia, in mixed deciduous forest and village hedgerows, altitude up to 1,000 m [4, 6]

Production of crude drug

A. Cultivation

The soil best suited for this plant is a rich friable loam with high humus content and good drainage. It is generally propagated by shoot cutting which should be about 15 cm long. Cuttings are to be inserted into the soil in bamboo baskests or plastic bags to obtain rooted cuttings for planting in the field. The crop requires support for climbing and putting forth satisfactory growth. Live supports are best for this purpose and generally low-branching trees.

B. Harvest

The shoot of the age at least three years old should be selected for harvest.

C. Post harvest handling

Select the stems of about 1.0-1.5 cm in diameter, wash throughly then cut into segments of variable lengths or slanted slice into pieces of about 0.5-1 cm thickness and dry under the sun.

D. Packaging and preservation

The dried crude drug should be kept in tightly closed containers or baled with plastic or a gunny sack. Label the name of crude drug and the date of its harvest. Store in a dry room.

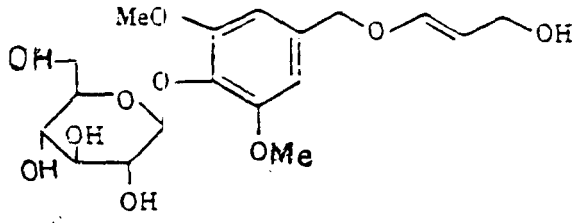
Part used

Fresh or dried stem

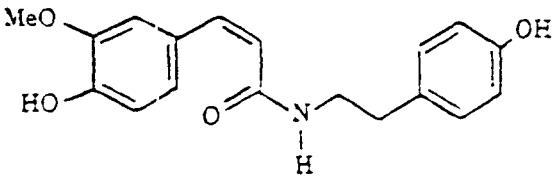
Chemical constituents

The stem contains :

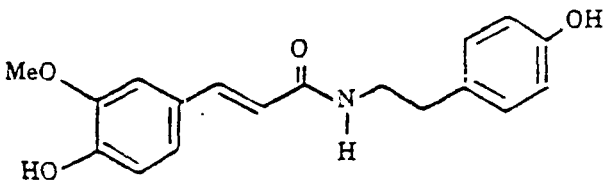
tinosporine, tinosporidine [12], picro-retin [13], N-transferuloyl tyramine, N-cis-feruloyl tyramine, **tinotuberide** [10], borapetoside A, borapetol A [11] ceryl alcohol, β-sitosterol, stigmasterol, and phytosterol [1, 10].



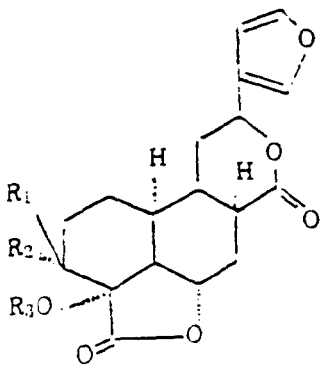
Tinotuberide



N-cis-feruloyl tyramine



N-trans-feruloyl tyramine



Borapetoside A : R_1 = -o-b-glc.pyr; R_2 = R_3 = H
Borapetol A : R_1 = OH; R_2 = R_3 = H

QUALITY SPECIFICATIONS

Official definition

Tinospora Caulis is the dried stem of *Tinospora crispa* **(L.) Miers ex Hook. F & Th** bitter activity is not less than 210 units/g.

Description

Odourless; taste intensely bitter

Identification

A. Pharmacognostic characteristics

Macroscopical

Dried whole or pieces of stem; externally brown, wrinkled, furrowed with characteristic warts; internally yellowish-white, radiate.

Microscopical

Tranverse and longitudinal sections of the stem show the following: Cork, several layers of rectangular brownish cells. Cortex, broad zone of parenchymatous cells containing starch grains; groups of sclereids containing prismatic crystals, occur beneath cork layers; parenchymatous cells containing prismatic crystals occur in the innermost part of cortex adjacent to phloem fibers. Stele composed of phloem and xylem separated by cambium, occurs several bands from cortex to pith, between the bands is medullary ray; phloem composed of thick walled fibers and phloem tissue; cambium, several layers of rectangular cells; xylem composed of large size of vessels, xylem fibers and non-lignified xylem parenchymatous cells containing starch grains; vessels, annular, spiral, recticulate, pitted and bordered pitted. Pith, containing larger size of parenchymatous cells.

Powdered drug

Greenish-brown odourless and intensely bitter.

Diagnostic structures :

Fragments of thin-walled parenchymatous cells containing starch grains; fragments of cork in surface view; fragments of xylem parenchymatous cells containing prismatic crystals; fragments of vessels or vessels associated with other xylem elements; fragments of fibers; scattered sclereids and stone cells, occasionally containing prismatic crystals; scattered prismatic crystals; scattered starch grains; 5-20 μm in size, pieces of phloem cells, rarely seen.

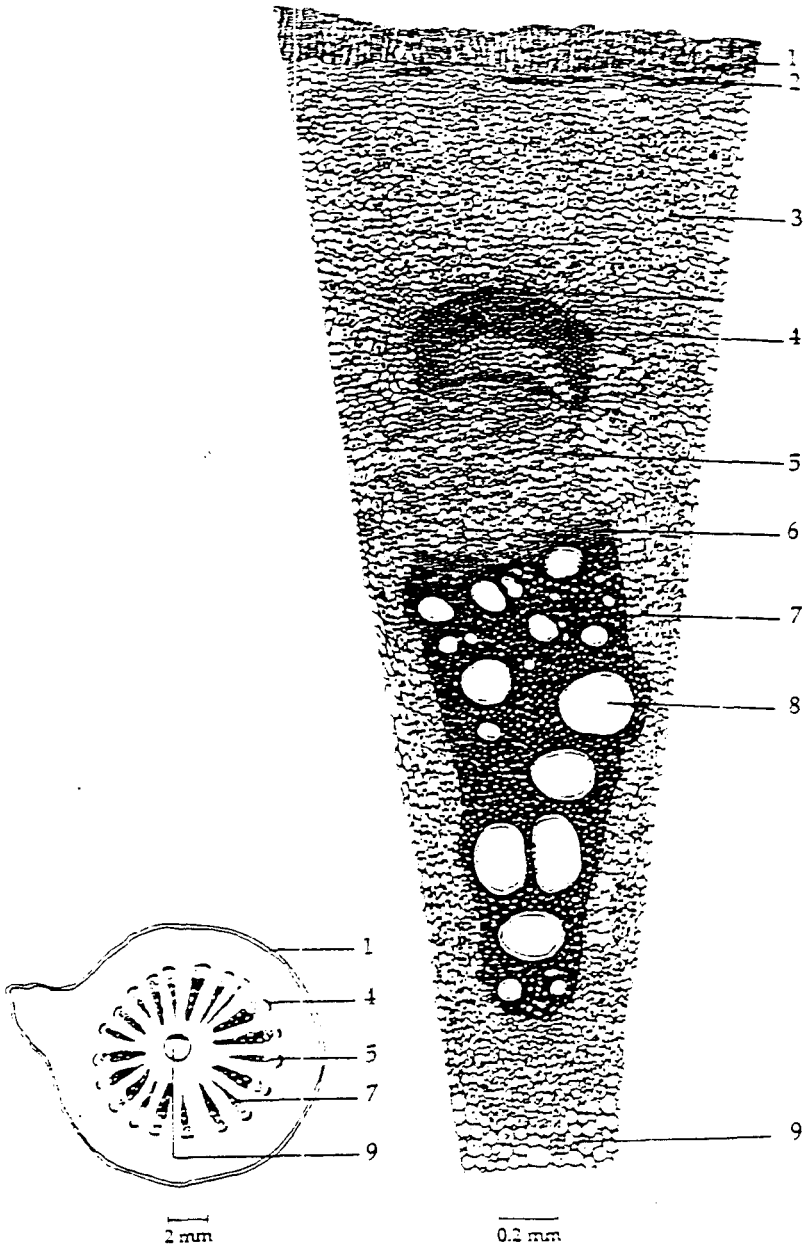


Fig. 2a Transverse Section of the Stem of *Tinospora crispa* (Linn.) Miers ex Hook. f. et Thoms.

1. cork
2. sclereid
3. cortical parenchyma
4. fibre
5. phloem
6. cambium
7. xylem fibre
8. vessel
9. pith

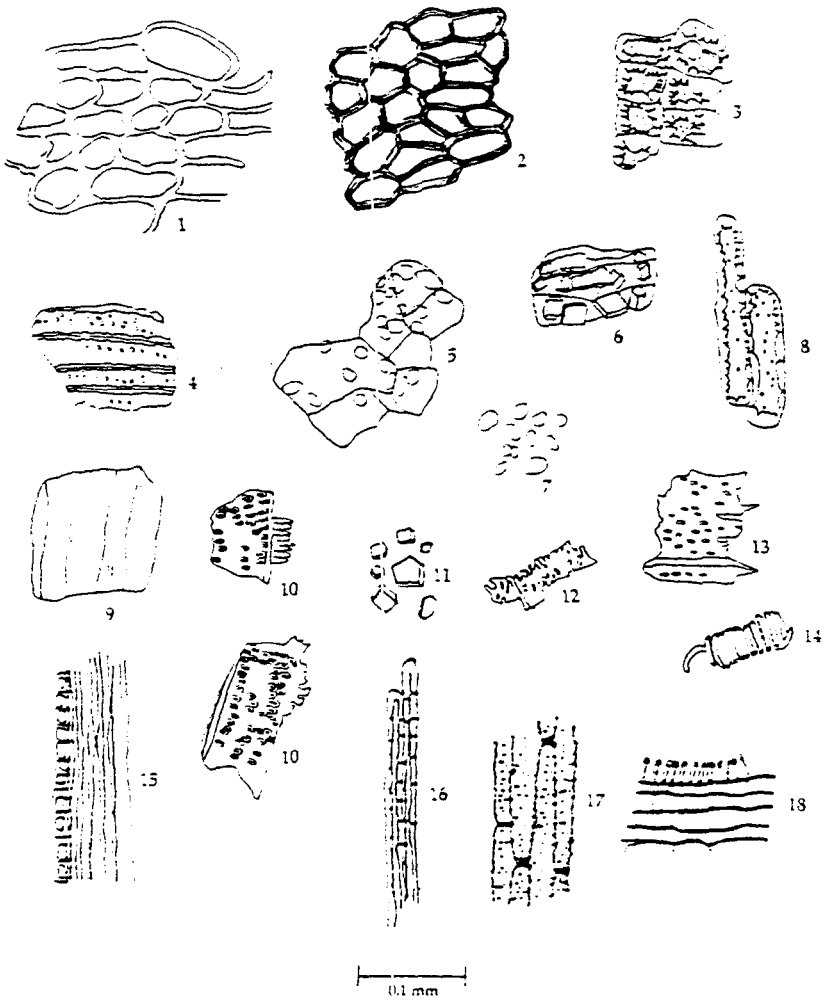


Fig. 2b Powdered Drug of the Stem of *Tinospora crispa* Miers ex Hook. f. et Thoms.

1. cortical parenchyma
2. cork in surface view
3. stone cells
4. xylem parenchyma
5. parenchyma cells with starch granules
6. parenchyma cells with prismatic crystals
7. starch granules
8. sclereids
9. phloem cells
10. bordered pitted vessels
11. prismatic crystals
12. reticulate vessel
13. pitted vessel
14. spiral thickening
15. parenchyma cell containing calcium oxalate crystals adjacent to bast fibres
16. fragments of bast fibres with dentated wall
17. fragments of lignified parenchyma
18. fragments of annular vessel with parenchyma cells

B. Chemical identification tests

Preliminary test

1. Shake vigorously 0.2 g of powdered drug with 10 mL of water. a long lasting foam is produced.
2. Extract 1 g of powdered drug with 10 mL of methanol on a water bath for 10 min, cool and filter. To 1 mL of the filtrate, add a few drops of Dragendorff reagent, an orange precipitate is formed.
3. Warm 0.5 g of powdered drug with 2 mL of acetic anhydride on a water bath for 2 min, filter. To the filtrate, add carefully 1 mL of sulfuric acid to make two layers. A brownish red colour forms at the zone of contact.

Confirmatory test

Sample solution :

Extract 1 g of powdered drug with 10 mL of methanol at 60° on a water bath for 10 min, filter, and the filtrate is evaporated to a volume of 2 mL.

Adsorbent : TLC plate silica gel **G**

Developing solvent

1. Hexane-ethylacetate-acetic acid (75:25:1)
2. Chloroform-methanol-ammonium hydroxide (75:20:5)

Developing distance : 12 cm

Spotting amount : 20 µL

Spray reagent :

1. Phosphomolybdic acid reagent
2. Dragendorff 's reagent or Acetic Potassium Iodobismuthate TS
3. Iodoplatinate reagent

Detection

1. Observe under UV 366 nm
2. Observe in daylight after treatment with phosphomolybdic acid reagent
3. Observe in daylight after treatment with Dragendorff 's reagent.
4. Observe in daylight after treatment with iodoplatinate reagent.

Table 1 hR_f values of Components in Methanolic Extract of the Stem of *Tinospora crispa* (Linn.) ex Hook. f. et Thoms.

Spot	hR_f value	Detection with			
		Mobile phase I		Mobile phase II	
		UV 366 (colour)	*Phosphomolybdic Acid* (colour)	*Acetic Potassium Iodobismuthate TS* (colour)	*Iodoplatinate TS* (colour)
1	1-3	light blue	dark blue	–	–
2	4-7	carmine red	–	–	–
3	9-12	moss green	–	–	–
4	12-17	light blue	–	–	–
5	14-16	–	–	orange	purple
6	20-24	light blue	–	–	–
7	25-29	carmine red	–	–	–
8	31-34	red	–	–	–
9	37-40	–	–	orange	purple
10	42-45	–	dark blue	–	–
11	45-50	red	–	–	–
12	49-51	–	–	orange	purple
13	51-55	carmine red	dark blue	–	–
14	55-58	–	dark blue	–	–
15	59-63	light blue	–	–	–
16	61-64	–	dark blue	–	–
17	86-90	–	dark blue	–	–
18	91-94	–	dark blue	–	–
19	92-96	–	–	–	orange
20	95-98	yellow	dark blue	–	–
21	96-98	–	–	moss green	moss green
22	98-99	–	–	yellow	yellow

Mobile phase I: 75 volumes of *hexane*, 25 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid*.

Mobile phase II: 75 volumes of *chloroform*, 20 volumes of *methanol* and 5 volumes of *strong ammonia solution*.

Bitter activity

Not less than 210 units g

Determination of bitter activity

Preparation of the stock of quinine hydrochloride and its dilutions : Dissolve accurately weighed 0.100 g of quinine hydrochloride in water to volume of 100.0 mL. Dilute 5 mL of this solution with water to volume of 500 mL. This solution is used as the stock solution of quinine-hydrochloride (Sq) containing 0.01 mg per mL.

Prepare a serial dilution of Sq in 9 test-tubes according to the following table for the first series of testing.

No. of tubes	1	2	3	4	5	6	7	8	9
ML of Sq.	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
ML of water	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Mg of quinine hydrochloride in 10 mL solution (c)	.042	.044	.046	.048	.050	.052	.054	.056	.058

Preparation of the stock solution of the drug to be tested and its dilutions : Accurately weigh 0.2 g of the powdered drug and place it into a 100 mL conical flask, add 45 mL of water, reflux in a boiling water bath for 1 hr with frequent shaking. Cool, filter through a corrugated funnel into a 50 mL volumetric flask. Add sufficient water through the funnel to bring the filtrate in the flask up to the mark. Pipette 1 mL of this extract into a 100 mL volumetric flask and dilute with water up to the mark. This is used as the stock solution of the drug to be testes (S_T). Calculate its concentration and express it in mg per mL.

Prepare a serial dilution of S_T in 10 test-tubes according to the following table for the second series of testing.

No.of tubes	1	2	3	4	5	6	7	8	9	10
mL of S_T(=b)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
mL of water	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	-

$$\text{Bitter activity} = \frac{2000 \times \mathbf{c}}{\mathbf{a} \times \mathbf{b}} \text{ units/g}$$

a = mg of drug contained 1 mL of S_T

b = mL of S_T contained in 10 mL of solution of threshold bitter concentration

c = mg of quinine hydrochloride contained in 10 mL of the solution of threshold bitter concentration

Foreign matter.

Not more than 2%

Ash contents

Total ash content : Not more than 7%

Acid-insoluble ash content : Not more than 0.5%

Extractives

Water-soluble extractive : Not less than 10%

Ethanol-soluble extractive : Not less than 5%

Loss on drying.

Not more than 8%

Indications

Antipyretic, stomachic and tonic [7]

Toxicity

Practically non toxic [8, 9]

Preparation used and dose[7]

Antipyretic, stomatic and bitter tonic.

Cut 25-30 g of dried stem or 30-40 g of fresh one, add 3 glasses of water, boil until 1 glass of decoction is obtained, and strain. Take ½ glass of decoction, twice a day before meal.

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4. J. Bansiddhi and D. Pecharaply, *Botanical Report of Some Thai Medicinal Plants*, part.1, Department of Medicinal Sciences, Bangkok,1988, p. 28-30.
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8. M.Mokkhasmit et al., *Bull. Dept. Med. Sci.* Thailand, 1971, 12 (2) : 36.
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13. R. Paris and L. Beauquesne, *Bull. Sci. Pharmacol.*, 1939, 46:73.
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WORKSHOP 1

Extraction and Purification of Tinotuberide from *Tinospora crispa*

Introduction

The stem of *Tinospora crispa* is one of the most popular traditional drugs in Thailand and other Southeast Asian countries, and is used as an appetizer, as antipyretic for malaria , and as a remedy for many other purposes. It is also phamacologically proven to be an antipyretic. For these reasons, The Government Pharmaceutical Organization has developed the crude drug into a conventional pharmaceutical dosage form to substitute for imported chemical drugs.

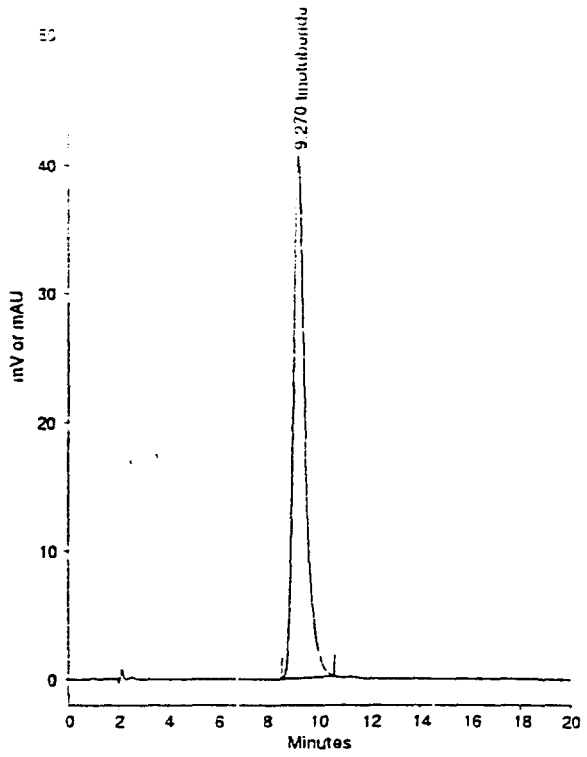
Consistent therapeutic efficacy of herbal drugs depends on consistent quality of finished products. Therefore, the control tests on raw materials and finished products (in terms of quality specification) are required to ensure the reproducibility of quality. In addition to the quality specification of Tinospora stem described in Thai herbal Pharmacopoeia and Standard of ASEAN herbal medicine, a more convenient qualitative and quantitative determination of Tinotuberide using HPLC was developed. Tinotuberide, a chemically defined marker was isolated and purified from the butanol extract. It has proved to be an excellent marker because it can be easily identified and quantified by HPLC method.

ISOLATION AND PURIFICATION OF TINOTUBERIDE

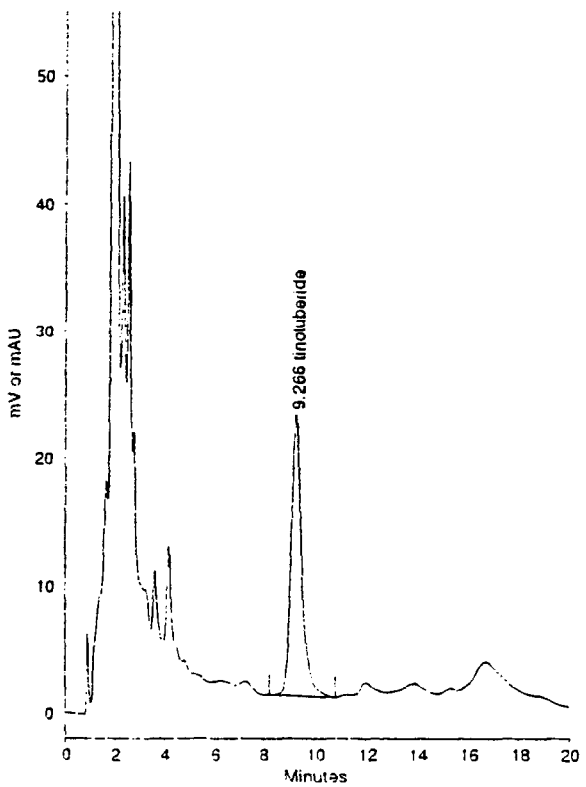
The stems were powdered and exhaustively extracted with methanol. The combined methanolic extract was evaporated under reduced pressure. The methanolic extract was dissolved in water and the solution was partitioned successively with hexane, ethyl acetate and butanol. The butanol extract was chromatographed over a silica gel column and eluted with mixed solvent ($CHCl_3$/MeOH/H_2O). The fraction containing a major constituent, tinotuberide, was combined and then rechromatographed over a silica gel column and eluted with different porportion of mixed solvent to yield tinotuberide. Further purification of tinotuberide was achieved by means of recrystallization.

QUANTITATIVE DETERMINATION OF TINOTUBERIDE IN CRUDE DRUG

1. Weigh 5 g of powdered stems of *Tinospora crispa* in a 250 ml round-bottomed flask with a ground glass neck.
2. Add 100 ml of water, weigh, connect the flask to a reflux condenser and heat in a water bath for 1 hr.
3. Allow to cool, weigh and restore the original weight with water.
4. Centrifuge, transfer 20 ml of supernatant to a 25 ml volumetric flask.
5. Adjust to volume with methanol and then mix.
6. Filter the solution through 0.45 μ membrane filter.
7. Analyze the quantity of Tinotuberide in the filtrate by using HPLC.

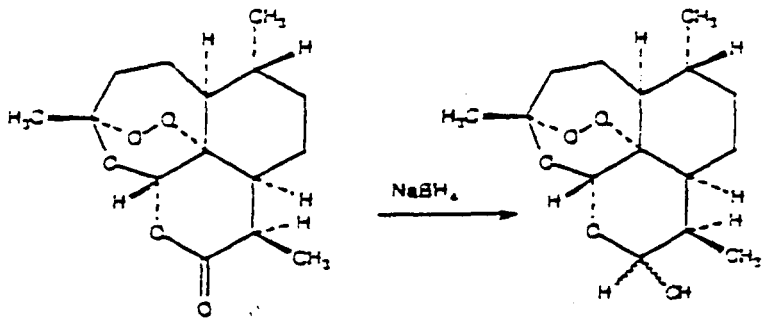


HPLC chromatogram of tinotuberide



HPLC chromatogram of tinotuberide in water extract

Chemical Modification of Artemisinin to dihydroartemisinin



Artemisinin (1) dihydroartemisinin (2)

MW 282 MW 284

Artemisinin (1; 0.5 g, 1.8 mmol) in 40 mL of MeOH was cooled in an ice bath to 0-5 °C. To the solution was added in small portions 0.25 g(6.6 mmol) of $NaBH_4$ over a period of 30 min. The solution was stirred at 0-5 °C for 2 hr after the addition of $NaBH_4$ was complete, and the solution was neutralized with 30% AcOH/MeOH and evaporated to dryness under reduced pressure. The white residue was extracted three times with 50 mL of EtOAc. The EtOAc extracts were combineds, filtered, and evaporated to dryness to give 0.38 g (75%) of white needles, mp 152-154 °C. Recrystallization from EtOAc/hexane raised the melting poin: to 153-155 °C (lit, mp 153-154 °C).

ref. 1) A.J. Lin, D.L. Klayman, and W.K. Milhous, J. Med. Chem., 30 , 2147 (1987)

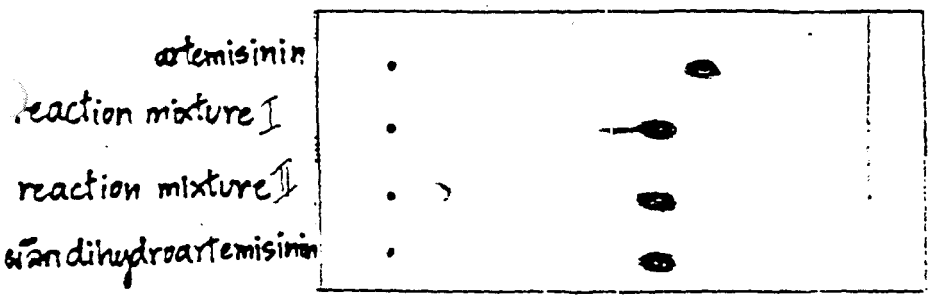
Thin-layer Chromatogram

Condition

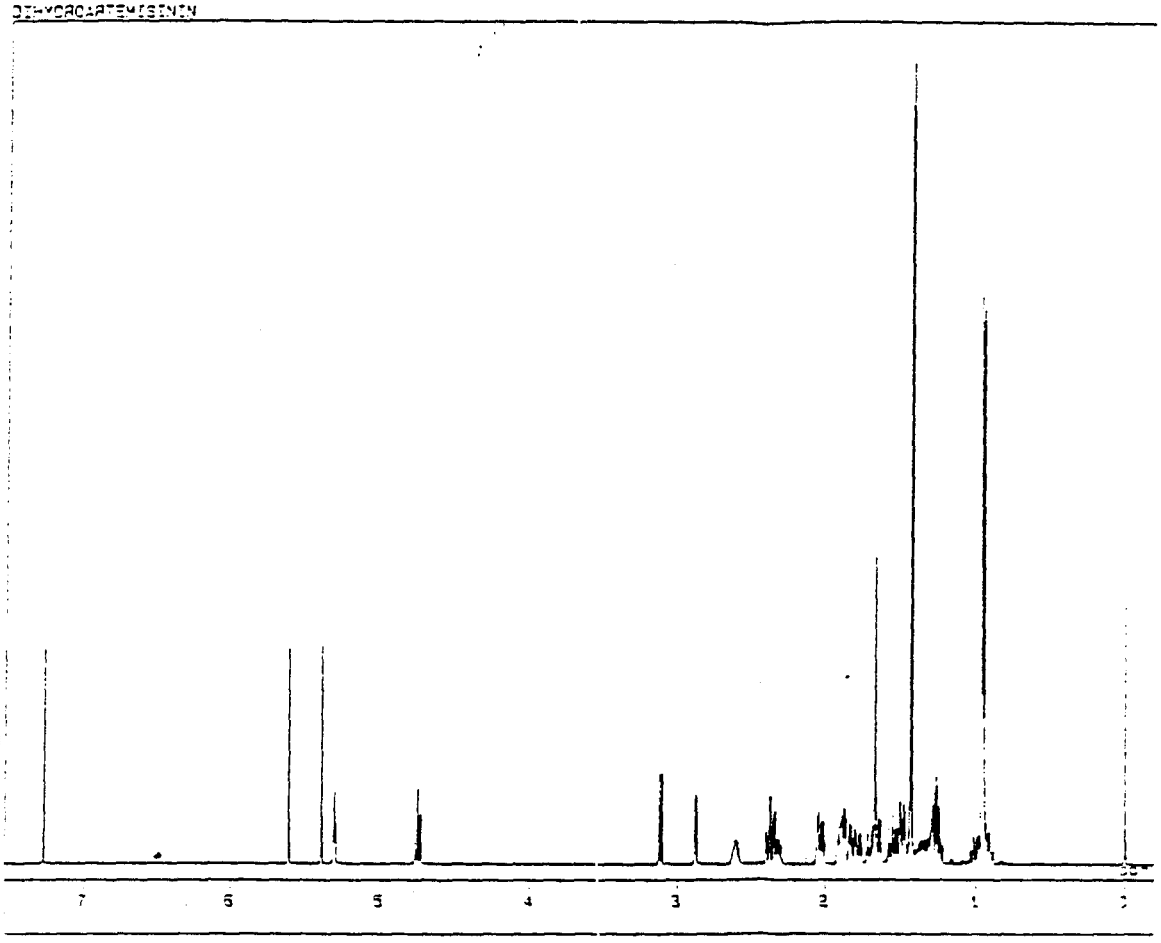
Stationary Phase : SiO_2 GF 254

Mobile Phase : n-Hexane : Ethyl acetate 1:1

Spray reagent : anisaldehyde-sulfuric acid



DIHYDROARTEMISININ



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CHULALONGKORN UNIVERSITY
JNM-A500

FILE /EB1CHAM-1H
COMNT DIHYDROARTEMISININ

EXMOD SINGL
TMOD NON
POINT 32768
FREQU 10000.00 Hz
SCANS 8
DUMMY 4
D1 3.27768 sec
D2 3.00000 sec
D11 1m
D12 5.00 usec
D13 1H
IRNUC 1H
SWIDTH 500.00 kHz
FREQU 199497.74 Hz
RFLVL 120
PULSE 30.0 usec
PW90 8.00
HOLD 1
DS 16
SS 32
RGAIN 20
FB 32768
DFILT 1
DFORM 2
MFILT 5.000 Hz
RECPH 90.00 deg
FREQU 3999.99 Hz
SWIDTH 4000.00 Hz

OPERATOR

MASS SPECTRUM : (6 TO 7)
SAMPLE:DIHYDRO ARTERMISININ 13 JAN 94
NOTE :356/1 EI 70V 300UA CHAMB.TEMP. 150
BASE PEAK : M/E 43.0 INT. 446.0

M/E	RAW INT.	R.INT.	SIGMA(%)	
41.0	149.7	335.6	8.52	
43.0	446.0	1000.0	25.39	
44.0	62.0	139.0	3.53	
55.0	175.1	392.7	9.97	
67.0	61.9	138.8	3.52	
69.0	99.5	223.1	5.66	
71.0	71.9	161.1	4.09	
81.0	84.6	189.7	4.81	
93.0	65.0	145.9	3.70	
95.0	85.8	192.4	4.88	
97.0	56.7	127.1	3.22	
101.0	53.6	120.2	3.05	
109.0	53.0	118.9	3.01	
110.0	51.5	115.6	2.93	
123.0	68.3	153.3	3.89	
137.0	87.8	197.0	5.00	
152.0	83.3	186.7	4.74	END

$M^+ - H_2O$

MASS SPECTRUM : (6 TO 7)
SAMPLE:DIHYDRO ARTERMISININ 13 JAN 94
NOTE :356/1 EI 70V 300UA CHAMB.TEMP. 150
BASE PEAK : M/E 43.0 INT. 446.0

M/E	RAW INT.	R.INT.	SIGMA(%)	
162.0	64.6	144.9	9.59	
163.0	53.4	119.9	7.93	
164.0	15.3	34.4	2.28	
165.0	15.6	35.1	2.32	
167.0	13.6	30.5	2.02	
173.0	8.9	20.1	1.33	
176.0	10.1	22.7	1.50	
177.0	28.4	63.8	4.22	
178.0	11.5	25.8	1.71	
179.0	15.5	34.7	2.29	
180.0	38.1	85.5	5.66	
181.0	20.0	44.8	2.96	
191.0	11.3	25.3	1.67	
194.0	98.6	221.2	14.63	
195.0	72.5	162.6	10.76	
196.0	10.9	24.6	1.62	
205.0	7.6	17.1	1.13	
206.0	9.1	20.4	1.35	
209.0	9.0	20.2	1.33	
210.0	28.4	63.7	4.21	
216.0	9.1	20.5	1.36	
219.0	8.3	18.6	1.23	
220.0	7.3	16.5	1.09	
223.0	8.6	19.4	1.28	
234.0	28.0	62.8	4.16	
237.0	16.7	37.6	2.48	
252.0	37.0	83.1	5.49	
266.0	15.2	34.1	2.25	END

BIOACTIVITY - GUIDED SCREENING OF SOME NATURAL PRODUCTS

Professor Haruhiro Fujimoto

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname : Fujimoto Other name : Haruhiro

Title (Mr, Mrs, Dr, Professor, etc) Dr.

Current Position : Associate Professor

Current Place of Work : Faculty of Pharmaceutical Sciences, Chiba University, Japan

Educational profile:

Year	Place of Study	Qualification	Field of study
1964	Chiba University	Bachelor	Pharmaceutical Sciences
1966	University of Tokyo	Master	Pharmaceutical Sciences
1970	University of Tokyo	Doctor	Pharmaceutical Science

Professional Experiences :

1968 Assistant, Research Institute for Foodmicrobiology, Chiba University

1972-1974 Postdoctoral Fellow, Miinster University, Germany

1975 Associate Professor, Research Institute for Chemobiodynamics, Chiba University

1987 Associate Professor, Faculty of Pharmaceutical Sciences, Chiba University

Current interests :

Structure and Activity of Natural Products Possessing Immunomodulatory and Neurotropic Activity

Bioactivity-Guided Screening of Some Natural Products

Haruhiro Fujimoto
(Faculty of Pharmaceutical Sciences, Chiba University,
1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan)

Bioactivity-guided screening of natural products is very important to development of medicines, health foods, cosmetics, agricultural chemicals, insecticides, and so on. Many compounds which have been found out by bioactivity-guided screening from natural material are today utilized as very useful medicines. In the screening, many kinds of bioactivity are utilizable, among which some are described below.

1. Stimulating or suppressive effect on the nervous system

- The nervous system
  - Central nervous system — Cerebrum, Brainstem, Spinal cord
  - Peripheral nervous system
    - Somatic nervous system
      - Motor nerve (efferent)
      - Sensory nerve (afferent)
    - Autonomic nervous system
      - Autonomic nerve (efferent)
        - Sympathetic nerve
          - Preganglionic neuron (cholinergic)
          - Postganglionic neuron (adrenergic)
        - Parasympathetic nerve
          - Preganglioic neuron (cholinergic)
          - Postganglionic neuron (cholinergic)
      - Viscerosensory nerve (afferent)

Both stimulating (accelerative, excitatory) and suppressive (inhibitory, blocking) effects on each nervous system, nerve, and neuron are present. Each effect is further fractionalized into many more specialized ones.

(For example) suppressive effect on central nervous system→analgesic effect, antipyretic effect, sedative effect, antianxious effect, etc.

2. Stimulating or suppressive effect on the immune system

The immune system

Cellular immunity (phagocytosis)

Humoral immunity (antigen-antibody reaction)

Both stimulating (recovery) and suppressive (inhibitory) effects on each immunity are present. Each effect is further fractionalized into several more specialized ones.

(For example) immunosuppressive effect→inhibitory effect on T-cell activation, immunocytolytic effect, antiallergic effect, etc.

3. Inhibitory effect on the activation of various enzymes

(For example) cyclooxigenase (COX) inhibitory effect (inhibition of prostagrandins and thromboxanes biosynthesis); angiotensin-converting enzyme inhibitory effect (inhibition of angiotensin II biosynthesis); hydroxymethylglutaryl (HMG)-CoA reductase inhibitory effect (inhibition of biosynthesis of sterols including cholesterol); monoamine oxidase (MAO) inhibitory effect (inhibition of the neurotransmitter, catecholamines metabolism); γ-aminobutyric acid (GABA) transaminase inhibitory effect (inhibition of the suppressive neurotropic amino acid, GABA metabolism); calcineurin inhibitory effect (inhibition of interleukin 2 (IL-2) biosynthesis), etc.

4. Cytotoxic effect on the growth of cultural cells

antitumor or carcinostatic effect on the growth of various cultural tumor or cancer cells (screening of antitumor or carcinostatic substances)

5. Antibacterial or antifungal effect on the growth of various bacteria or fungi which cause infectious diseases (screening of antibiotic substances)

6. Antagonistic effect on the activity of bioactive substances

(For example) opioid (morphine)-like effect on the response of guinea pig ileum preparation due to the morphine antagonist, naloxone (antagonism between an agonist and its antagonist on a receptor)

7. Recovery effect on the functional abnormality of various organs

recovery (cure, healing, remedy) effects on liver functional abnormality (disease); on kidney functional abnormality; on blood functional abnormality, etc.

8. Other bioactive effects on mammals (neurotransmitter-like effect, hormone-like effect, autacoid-like effect, etc.)

9. Bioactive effects on plants [phytohormone (plant hormone) effect, phytoalexin effect, allelopathy effect, etc.]
(For example) phytohormone effect: promotive effect on the growth of avena (avena curvature test, avena straight growth test); phytoalexin effect: suppressive effect on the spore germination of plant pathogenic fungi; allelopathy effect: suppressive effect on the growth of plant seedling

10. Bioactive effects on insects (transformation hormone effect, juvenile hormone effect, pheromone effect, etc.)
(For example) transformation hormone effect: emergence effect on a brain-ectomized pupa of silkworm; juvenile hormone: suppressive effect on transformation of corpora allata-ectomized larva of silkworm into pupa; pheromone effect: attractive effect on the opposite sexual insect

As the natural material for bioactivity-guided screening, a) crude drugs or traditional drugs, utilized by various races in the world, which possess already some medicinal informations, b) plants which have taxonomically close relation to the origin plants of the useful crude drugs, and c) plants and microorganisms which have taxonomically close relation to the plants and microorganisms containing the useful constituents, respectively, have more advantage than d) plants and microorganisms which give no information about their constituents.

On the workshop, the results of immunosuppressive activity-guided screening and monoamine oxidase inhibitory activity-guided screening of some new fungal metabolites which were recently executed in our laboratory will be mainly talked.

Evaluation of Immunomodulatory Activity

BALB/c male mouse

↓

Spleen

↓ homogenized in RPMI1640 medium

Cell Suspension

← Ficoll-Urografin

↓ centrifuged at 1500 rpm for 20 min at 20℃

Supernatant

↓ centrifuged at 1000 rpm for 5 min at 4℃ x 3

Precipitation

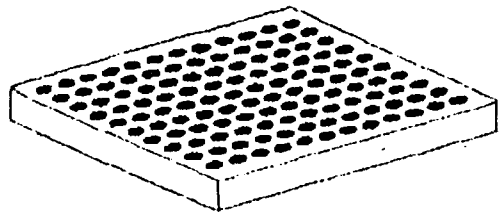
↓ dissolved in cRPMI

Lymphocyte Soln.
(8.0×10^6 cell/ml)

Lymphocyte Soln. 50 μl

Mitogen in cRPMI 50 μl

Sample in 1%EtOH/cRPMI 100 μl



(Microtiterplate)

↓ incubated at 37℃ under 5%CO_2 for 72 hr

← MTT in PBS-(-) (5 mg/ml) 20 μl

↓ incubated at 37℃ under 5%CO_2 for 4 hr

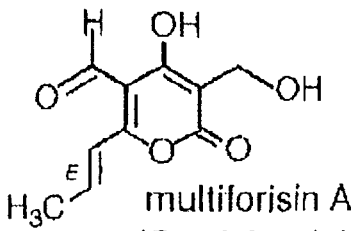
↓ centrifuged at 1000 rpm for 5 min at 4℃

Precipitation

↓ dissolved in DMSO

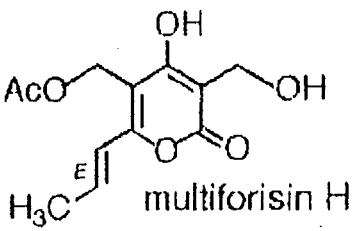
Measurement of Optical Density ($OD_{570nm}-OD_{630nm}$) by Microplate Reader

Some Immunosuppressive Components Isolated from Fungi in Our Laboratory



multiforisin A[1]

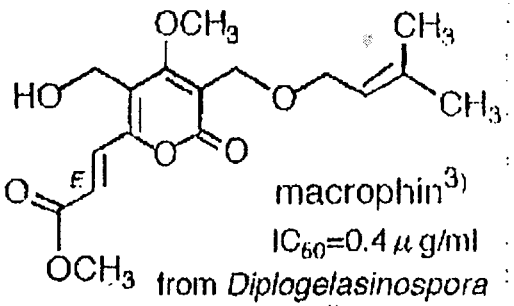
IC_{50}=0.6 μg/ml



multiforisin H[2]

IC_{50}=1.8 μg/ml

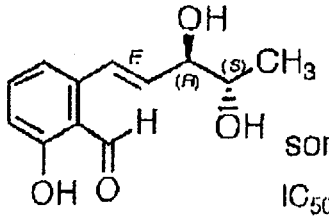
from *Gelasinospora multiforis, G.heterospora, G.longispora*



macrophin[3]

IC_{50}=0.4 μg/ml

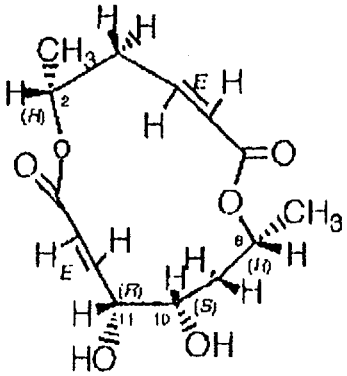
from *Diplogelasinospora grovesii*



sordarial[2]

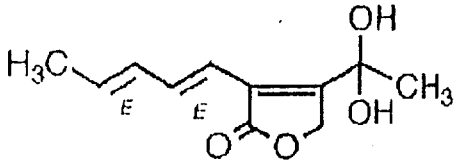
IC_{50}=6.5 μg/ml

from *Gelasinospora heterospora, G.longispora*



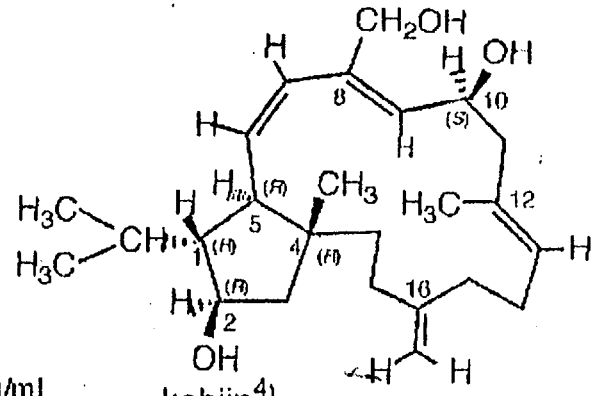
10-*epi*-colletodiol[3] IC_{50}=5.0 μg/ml

from *Diplogelasinospora grovesii*



kobifuranone C[4] IC_{50}=13 μg/ml

from *Gelasinospora kobi*



kobiin[4]

IC_{50}=7.0 μg/ml

from *Gelasinospora kobi*

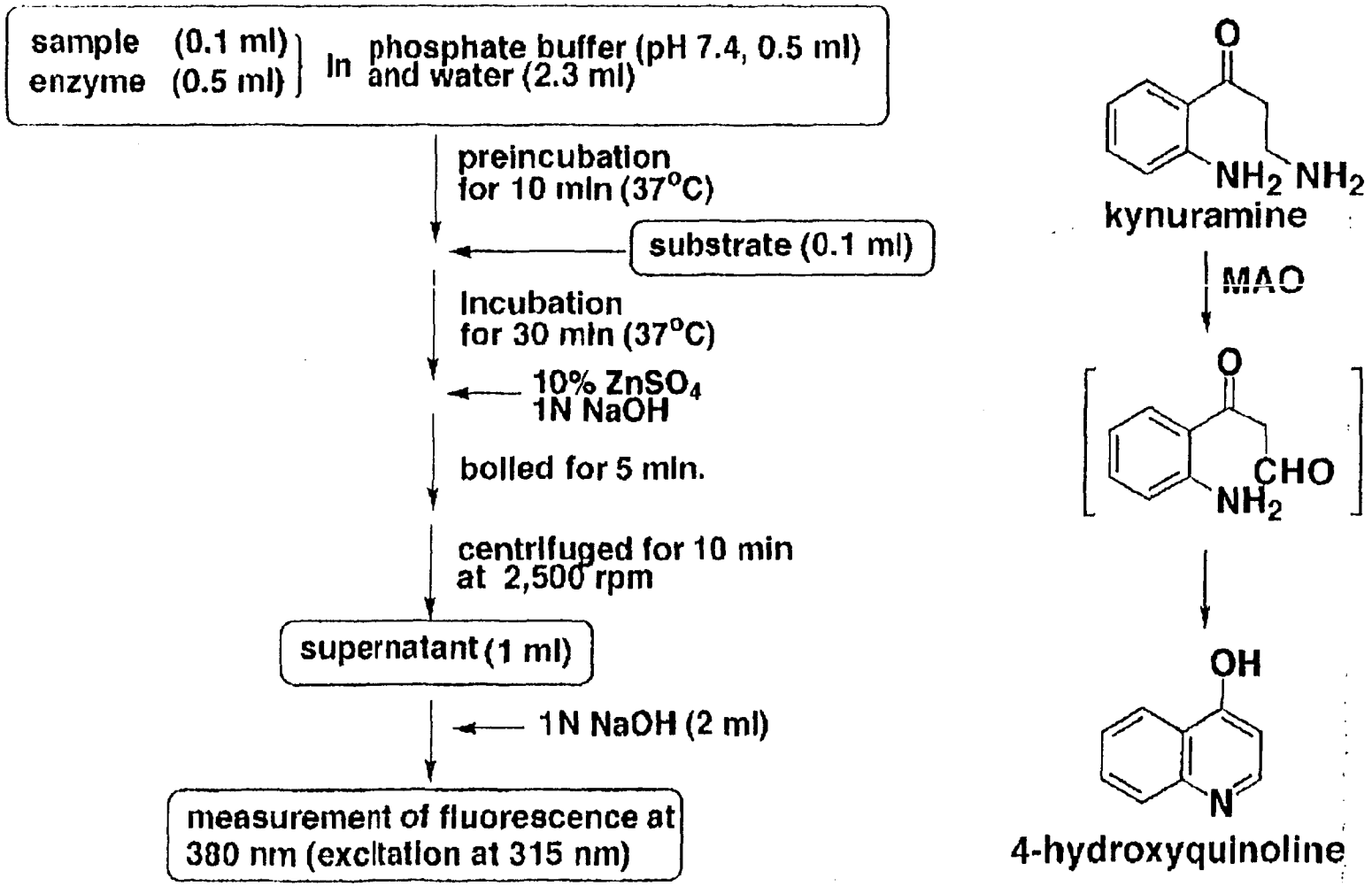
1) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **43**, 547 (1995)

2) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **47**, 71 (1999)

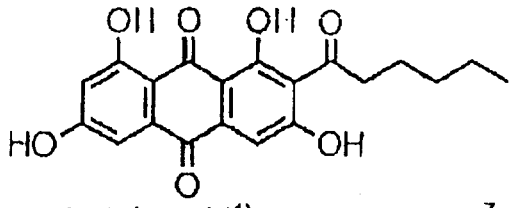
3) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **46**, 423 (1998)

4) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **46**, 211 (1998)

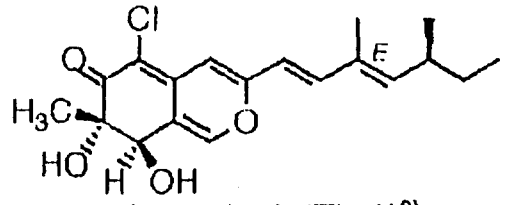
MAO Inhibition Assay: Modified Kraml's Method



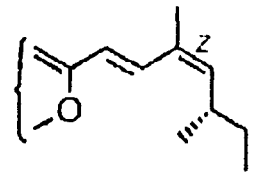
Some MAO-Inhibitory Components Isolated from Fungi in Our Laboratory



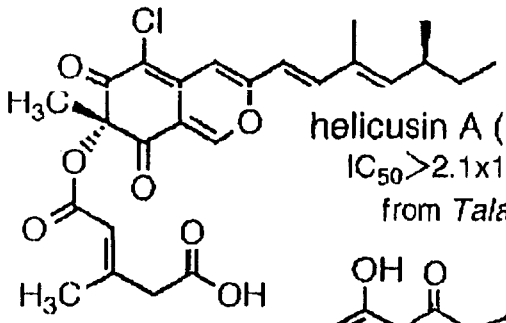
norsolorinic acid[1] $IC_{50}=3.0\times10^{-7}$M
from *Emericella navahoensis*



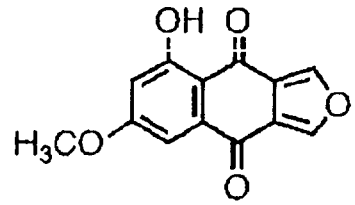
luteusin A (TL-1)[2] $IC_{50}=6.6\times10^{-6}$M



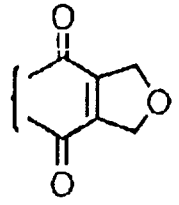
luteusin B (TL-2)[2] $IC_{50}=1.1\times10^{-5}$M
from *Talaromyces luteus*



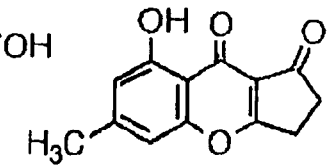
helicusin A (TH-1)[3]
$IC_{50}>2.1\times10^{-4}$M(1.0×10^{-4}g/ml)
from *Talaromyces helicus*



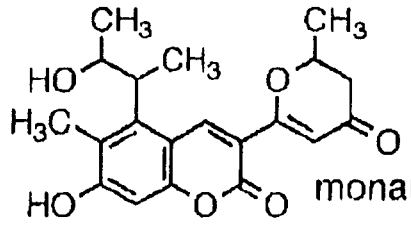
GP-A[4] $IC_{50}=2.7\times10^{-6}$M



GP-B[4] $IC_{50}=2.0\times10^{-6}$M
from Mycelia Sterilia of *Gelasinospora pseudoreticulata*



coniochaetone A[5] $IC_{50}=2.9\times10^{-5}$M
from *Coniochaeta tetraspora*



monankarin A[6] $IC_{50}=1.6\times10^{-5}$M
from *Monascus anka*

1) M.Yamazaki *et al.*, *Chem.Pharm.Bull.*, **36**, 670 (1988)
2) Y.Satoh *et al.*, *Chem.Pharm.Bull.*, **37**, 206 (1989); H.Fujimoto *et al.*, *Heterocycles*, **30**, 607 (1990)
3) E.Yoshida *et al.*, *Chem.Pharm.Bull.* **43**, 1307 (1995)
4) H.Fujimoto *et al.*, *Mycotoxins*, **41**, 61 (1995)
5) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **44**, 1090 (1996)
6) C.F.Hossain *et al.*, *Chem.Pharm.Bull.*, **44**, 1535 (1996)

Bioactivity-Guided Screening of Some Natural Products

Haruhiro Fujimoto

(Faculty of Pharmaceutical Sciences, Chiba University, Japan)

Bioactivity (1)

1. Stimulating or Suppressive Effect on the Nervous System

- **The Nervous System**
  - **Central Nervous System** — **Cerebrum, Brainstem, Spinal Cord**
  - **Peripheral Nervous System**
    - **Somatic Nervous System**
      - **Motor Nerve (Efferent)**
      - **Sensory Nerve (Afferent)**
    - **Autonomic Nervous System**
      - **Autonomic Nerve (Efferent)**
        - **Sympathetic Nerve**
          - **Preganglionic Neuron (Cholinergic)**
          - **Postganglionic Neuron (Adrenergic)**
        - **Parasympathetic Nerve**
          - **Preganglionic Neuron (Cholinergic)**
          - **Postganglionic Neuron (Cholinergic)**
      - **Viscerosensory Nerve (Afferent)**

Both stimulating (accelelative, excitatory) and suppressive (inhibitory, blocking) effects on each nervous system, nerve, and neuron are present. Each effect is further fractionalized into many more specialized ones.

(For example) Suppressive effect on central nervous system ⟶ analgesic effect, antipyretic effect, sedative effect, antianxious effect, etc.

Bioactivity (2)

2. Stimulating or Suppressive Effect on the Immune System

The Immune System

- **Cellular Immunity**
  - **Antigen Recognition by APC [Interleukin 1 (IL-1) biosynthesis]**
    - → **T-Cell Activation (IL-2 biosynthesis, Differentiation into Various Types of T-Cells, and Proliferation)**
      - → **NK-Cell and Macrophage Activation**
        - → **Phagocytosis, Allergy-IV**
- **Humoral Immunity**
  - **(Antigen Recognition by APC, T-Cell Activation)**
    - → **B-Cell Activation (Differentiation into Plasma Cell, and Proliferation)**
      - → **Antigen-Antibody Reaction, Allergy-I, -II, and -III**

Both stimulating (recovery) and suppressive (inhibitory) effects on each immunity stage are present, Each effect is further fractionalized into several more specialized ones.

(For example) Immunosuppressive effect → inhibitory effect on T-cell activation, immunocytolytic effect, antiallergic effect, etc.

3. Inhibitory Effect on the Activation of Various Enzymes

(For example) Cyclooxygenase (COX) inhibitory effect (Inhibition of prostagrandins and thromboxanes biosynthesis); Angiotensin-converting enzyme inhibitory effect (Inhibition of angiotensin II biosynthesis); Hydroxymethylglutaryl (HMG)-CoA reductase inhibitory effect (Inhibition of biosynthesis of sterols including cholesterol); Monoamine oxidase (MAO) inhibitory effect (Inhibition of the neurotransmitter, catecholamines metabolism); γ-aminobutyric acid (GABA) transaminase inhibitory effect (Inhibition of the suppressive neurotropic amino acid, GABA metabolism); Calcineurin inhibitory effect (Inhibition of IL-2 biosynthesis); Topoisomerases I and II inhibitory effects (DNA gyrase Inhibitory effect: screenig of carcinostatic substances); etc.

Bioactivity (3)

4. **Cytotoxic Effect on the Growth of Cultural Cells (Screening of Antitumor or Carcinostatic Substances)**

   Antitumor or Carcinostatic Effect on the Growth of Various Cultural Tumor or Cancer Cells

   (For example) Cytotoxic effects on human promyelocytic leukemia HL-60 cells, on mouse transplantation leukemia P388 cells, on mouse transplantation leukemia L1210 cells, on mouse transplantation Colon 26 cancer cells, etc.

5. **Antibacterial or Antifungal Effect on the Growth of Various Bacteria or Fungi Which Cause Infectious Diseases (Screening of Antibiotic Substances)**

   (For example) Antibacterial effect on *Candida albicans, Cryptococcus neoformans, Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, etc., and antifungal effect on *Aspergillus fumigatus*, etc.

6. **Antagonistic Effect on the Activity of Bioactive Substances (Screening of Antagonistic Substances on the Basis of Antagonism between an Agonist and its Antagonist on a Receptor)**

   (For example) Opioid (morphine)-like effect on the response of guinea pig ileum preparation due to the morphine antagonist, naloxone, etc.

7. **Recovery Effect on the Functional Abnormality of Various Organs**

   (For example) Recovery (cure, healing, remedy) effects on liver functional abnormality (disease), on kidney functional abnormality, on blood functional abnormality, etc.

8. **Other Bioactive Effects on Mammals**

   (For example) Screening of the substances possessing neurotransmitter-like effect, hormone-like effect, autacoid-like effect, etc.

Bioactivity (4)

9. Bioactive Effect on Plants [Phytohormone (Plant Hormone) Effect, Phytoalexin Effect, Allelopathy Effect, etc.]

(For example) Phytohormone effect: promotive effect on the growth of avena (*Avena sativa* L.) (avena curvature test, avena straight growth test), **Phytoalexin effect**: suppressive effect on the spore germination of plant pathogenic fungi, **Allelopathy effect**: suppressive effect on the growth of plant seedling

10. Bioactive Effect on Insects (Transformation Hormone Effect, Juvenile Hormone Effect, Pheromone Effect, etc.

(For example) Transformation hormone effect: emergence effect on a brain-ectomized pupa of silkworm, **Juvenile hormone effect**: suppressive effect on transformation of corpora allata-ectomized larva of silkworm into pupa, **Pheromone effect**: attractive effect on the opposite sexual insect

11. Fungicidal Effect on Some Plant Pathogenic Fungi

(For example) Fungicidal effects on the rice blast, *Pyricularia oryzae* and the Cucurbitaceae plant powdery mildew, *Sphaerotheca fuliginea* are tested by spray of sample solutions to rice seedling and Cucurbitaceae plant cotyledon before inoculations of these plant pathogenic fungi, and observations for 7 days, respectively

12. Insecticidal Effect on Some Plant Noxious Insects

(For example) Insecticidal effects on the rice planthoppers (the Delphacidae insects), *Sogatella furcifera*, *Nilaparvata lugens*, etc., the cabbage noxious insect, *Spodoptera litura*, and the bean two-spotted spider mite, *Tetranychus urticae* are tested by spray of sample solutions to rice seedling, a cabbage leaf disc in a plastic cup, and a green bean leaf disc on agar before exposures to these noxious insects, and observations for 3－7 days, respectively

Natural Material

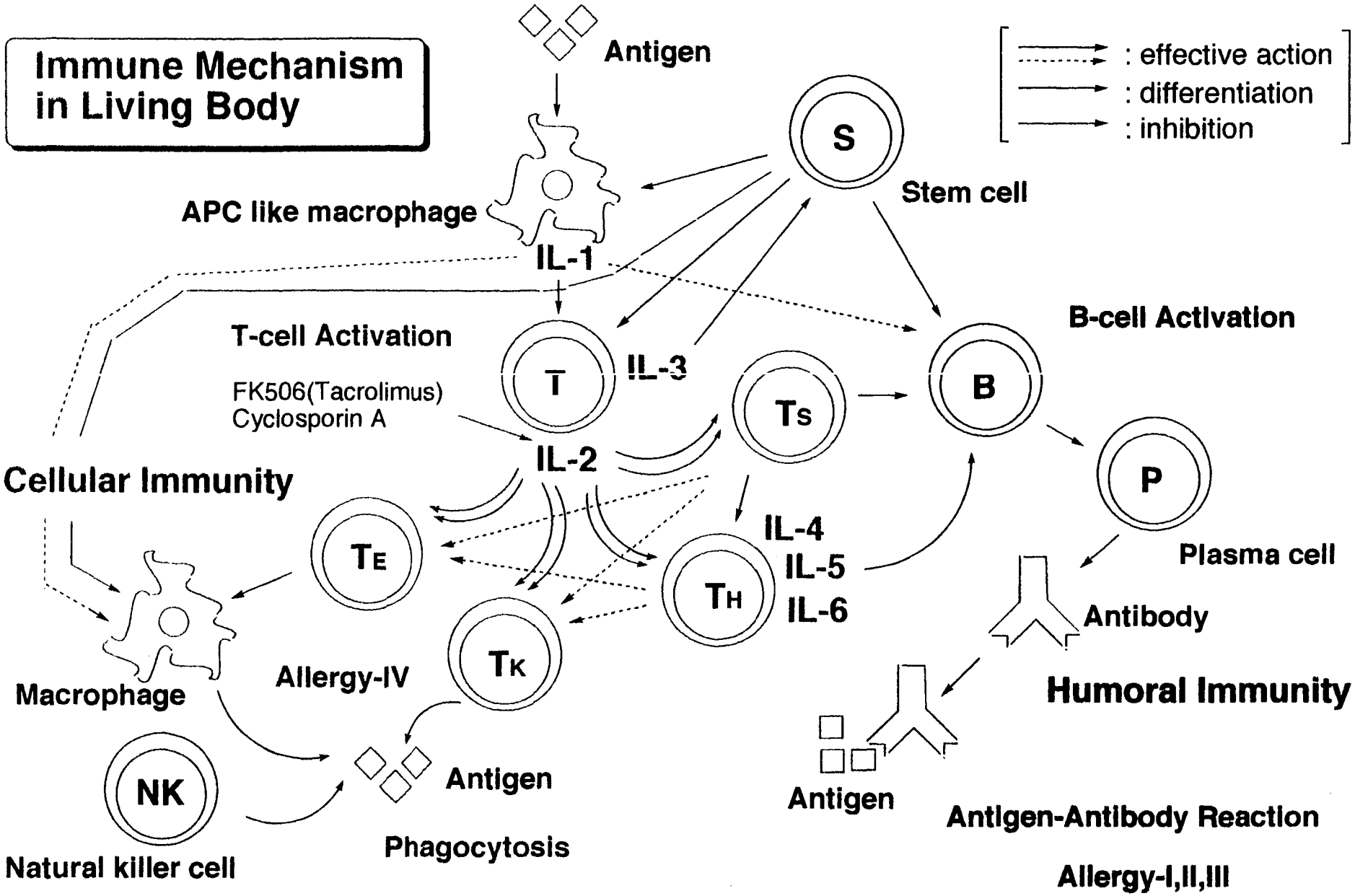
a) Crude Drugs or Traditional Drugs, Utilized by Various Races in the World, Which Possess Already Some Medicinal or Medical Informations

b) Plants Which Have Taxonomically Close Relation to the Origin Plants of the Useful Crude Drugs or Traditional Drugs

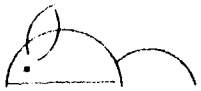
c) Plants, Microorganisms, and Marine Living Things Which Have Taxonomically Close Relations to the Plants, Microorganisms, and Marine Living Things Containing the Useful Constituents, Respectively

d) Plants, Microorganisms, and Marine Living Things Which Give no Information about Their Constituents

As the Natural Material for Bioactivity-Guided Screening Aiming at Development of Medicines, Health Foods, Cosmetics, Agricultural Chemicals, Insecticides, and So on,
Group a) Has the Greatest Advantage, Groups b) and c) Have
More Great Advantages than Group d).


Immune Mechanism in Living Body
Antigen
: effective action
: differentiation
: inhibition
S
Stem cell
APC like macrophage
IL-1
T-cell Activation
B-cell Activation
T
IL-3
FK506(Tacrolimus)
Cyclosporin A
Ts
B
IL-2
Cellular Immunity
P
Plasma cell
IL-4
IL-5
IL-6
TE
TH
Antibody
TK
Allergy-IV
Macrophage
Humoral Immunity
NK
Antigen
Antigen
Antigen-Antibody Reaction
Phagocytosis
Allergy-I,II,III
Natural killer cell

Evaluation of Immunomodulatory Activity



BALB/c male mouse

↓

Spleen

↓ homogenized in RPMI1640 medium

Cell Suspension

← Ficoll-Urografin

↓ centrifuged at 1500 rpm for 20 min at 20℃

Supernatant

↓ centrifuged at 1000 rpm for 5 min at 4℃ x 3

Precipitation

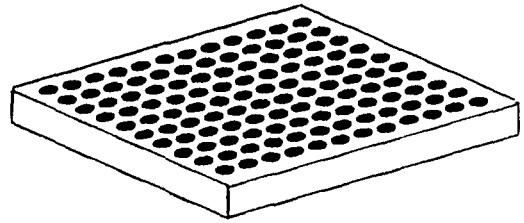
↓ dissolved in cRPMI

Lymphocyte Soln.
(8.0×10^6 cell/ml)

Lymphocyte Soln. 50 μl

Mitogen in cRPMI 50 μl

Sample in 1%EtOH/cRPMI 100 μl



(Microtiterplate)

↓ incubated at 37℃ under 5%CO_2 for 72 hr

← MTT in PBS-(-) (5 mg/ml) 20 μl

↓ incubated at 37℃ under 5%CO_2 for 4 hr

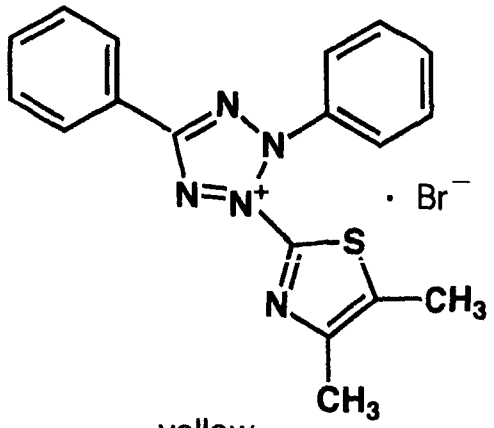
↓ centrifuged at 1000 rpm for 5 min at 4℃

Precipitation

↓ dissolved in DMSO

Measurement of Optical Density ($OD_{570nm}-OD_{630nm}$) by Microplate Reader

MTT Assay

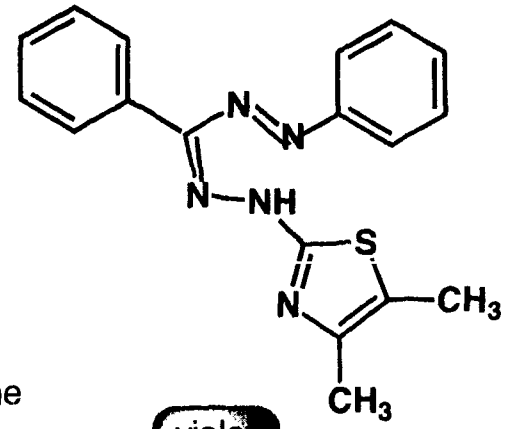


yellow

[H⁻]

[−H⁻]

enzyme in the respiratory chain
in living mitochodrial inner membrane



violet

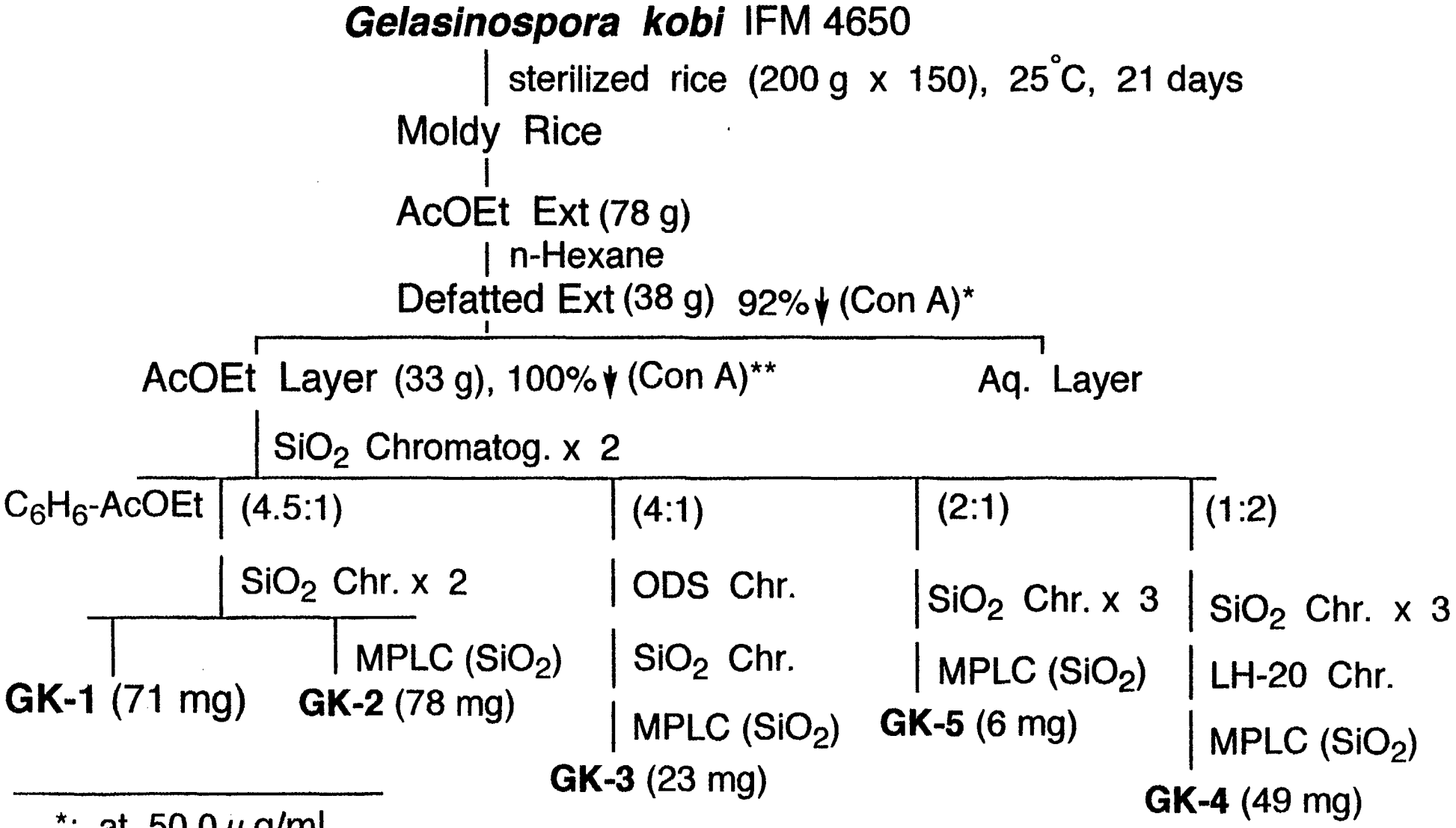
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-
2*H*-tetrazolium bromide (MTT)

3,5-diphenyl-1-(4,5-dimethylthiazol-
2-yl)-formazan (MTT formazan)

test wavelength : 570 nm
reference wavelength : 630 nm

T. Mosmann, J. Immunological Methods, **65**, 55-63 (1983)

Isolation of GK-1 - -5 from *G. kobi*



*: at 50.0 μg/ml

**: at 12.5 μg/ml

Physico-Chemical Property of GK-4 from *G. kobi*

pale yellow oil, $[\alpha]_D^{22}$ +41.2° ($CHCl_3$)

$C_{25}H_{40}O_3$ HRFAB-MS m/z: 427.2612 [$(M+K)^+$]

UV (MeOH) nm (log ε) : 238.4 (3.69)

IR ($CHCl_3$) cm^{-1} : 3600 - 3200 (O-H), 2930 (C-H), 1640, 1610 (C=C), 1380 (C-O)

NMR ($CDCl_3$) ^{1}H-NMR/*^{13}C-NMR* : δ (ppm)

4 CH_3- 0.85(s)/*24.96*, 1.00(d)/*22.47*, 1.02(d)/*22.28*, 1.66(s)/*24.96*

8 -CH_2- 1.26(m),1.49(m)/*40.75*, 1.68(2H,m)/*47.55*, 1.91(2H,m)/*31.88*,2.05(m),2.23(m)/*35.01*, 2.18(dd),2.49(br d)/*46.68*, 2.20(2H,m)/*27.17*, 3.93(d),4.31(d)/62.27, 4.72(s),4.74(s)/108.92

9 >CH- 1.49(t)/*56.50*, 1.80(m)/*27.94*, 3.02(t)/*46.16*, 4.28(td)/*73.86*, 4.63(ddd)/67.15, 5.21(t)/127.44, 5.46(t)/*135.81*, 5.74(d)/*132.13*, 5.92(d)/*128.90*

4 >C< *44.86, 130.55, 139.43, 150.70*

Ac_2O/Pyr, r.t. →

Triacetate (pale yellow oil),
δ 2.03, 2.04, 2.06 (each 3H, s), 4.67 (2H, s) (+0.74, 0.36), 5.25 (td) (+0.97), 5.75 (td) (+1.12) (in $CDCl_3$)

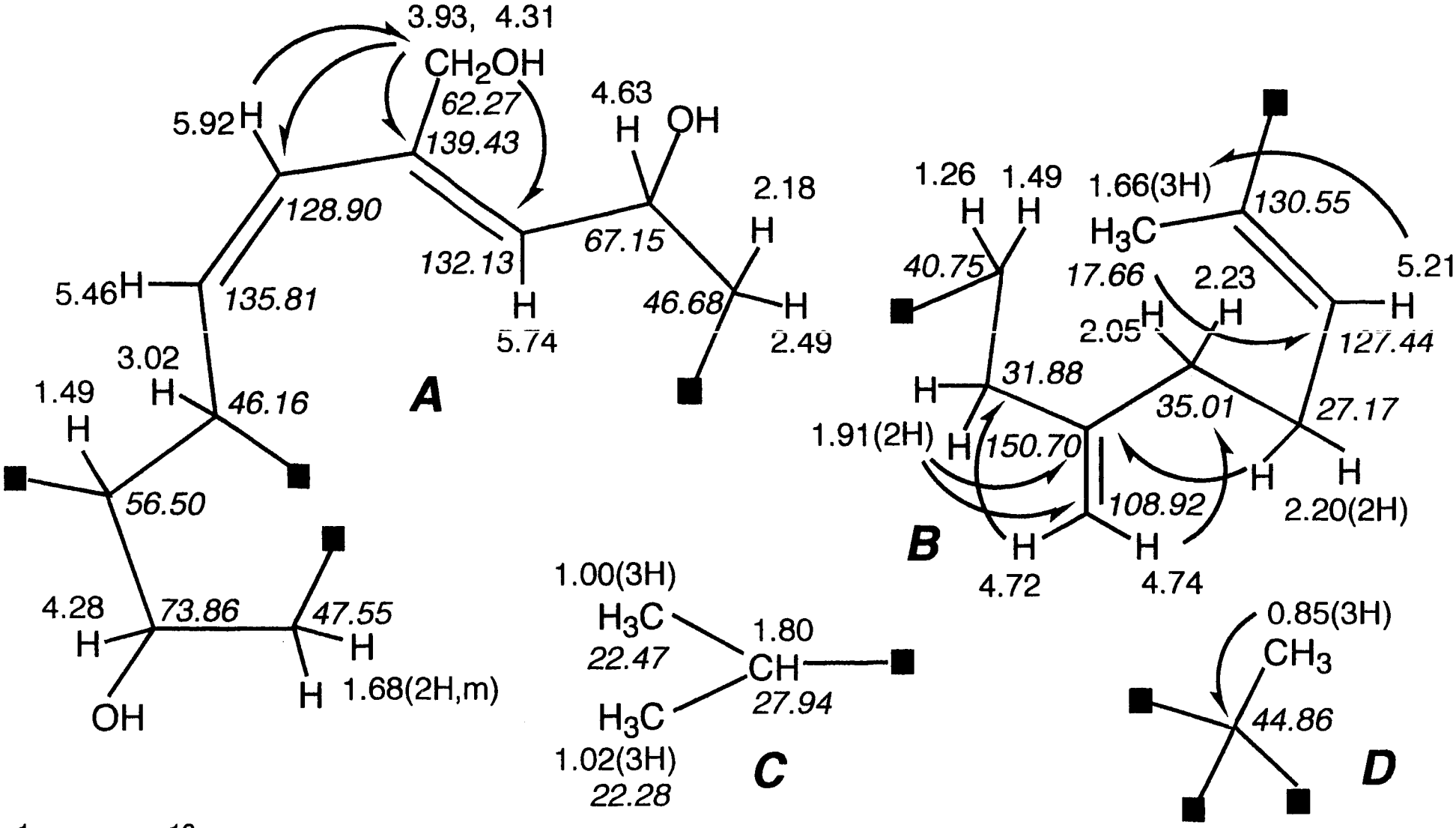
C_6H_5COCl/Pyr, r.t. →

Dibenzoate (pale yellow oil) + Tribenzoate (pale yellow oil)

δ 4.91, 5.03 (each 1H, d), 6.07(td), 7.32-8.00 (C_6H_5 x 2) (in $CDCl_3$)

δ 4.93, 5.06 (each 1H, d), 5.54 (td, 5.7, 1.7), 6.15 (td), 7.32-8.09 (C_6H_5 x 3) (in $CDCl_3$)

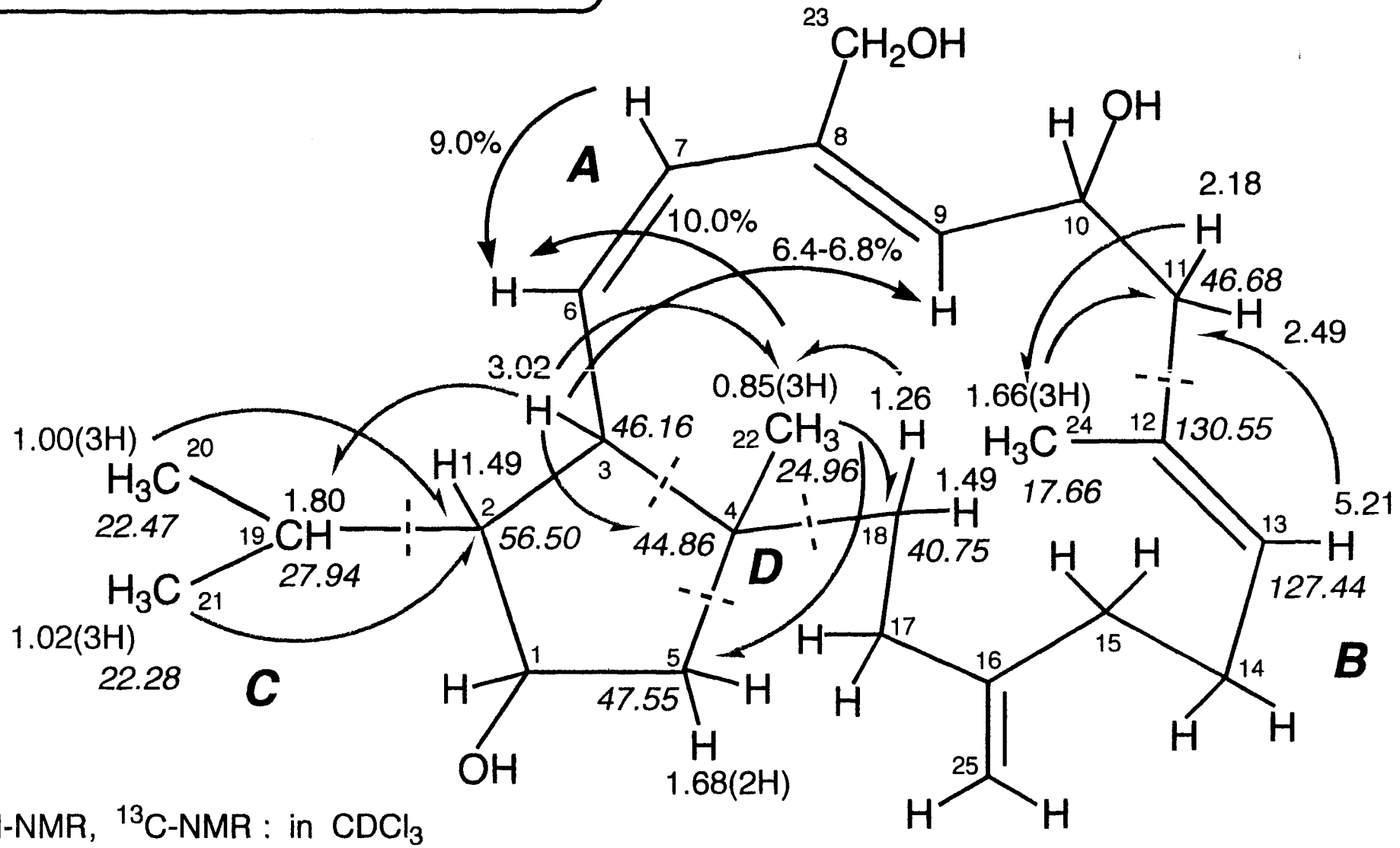
Partial Structures *A* - *D* for GK-4



^{1}H-NMR, *^{13}C-NMR*: in $CDCl_3$

: HMBC

Plane Structure of GK-4

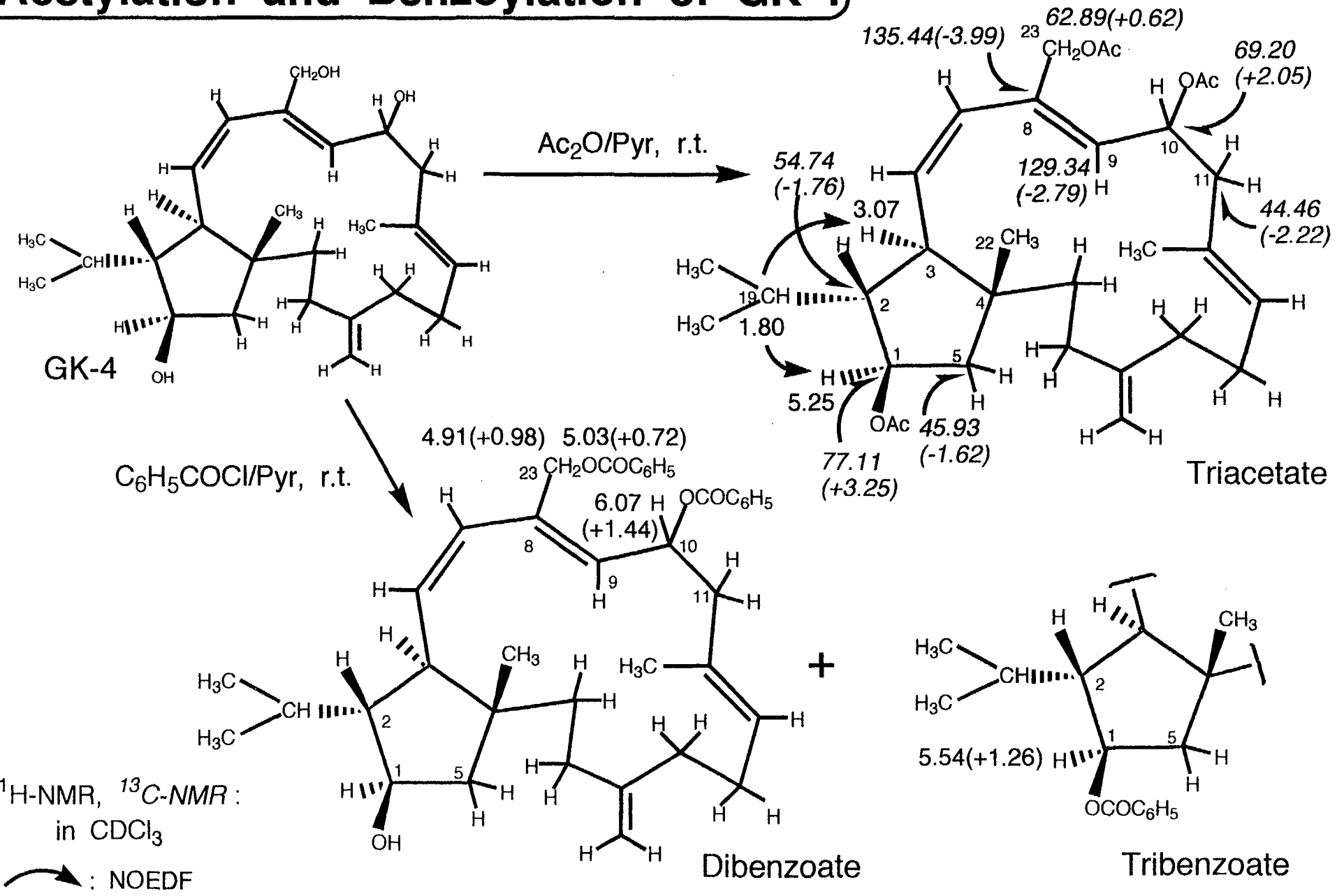


^{1}H-NMR, ^{13}C-NMR : in $CDCl_3$

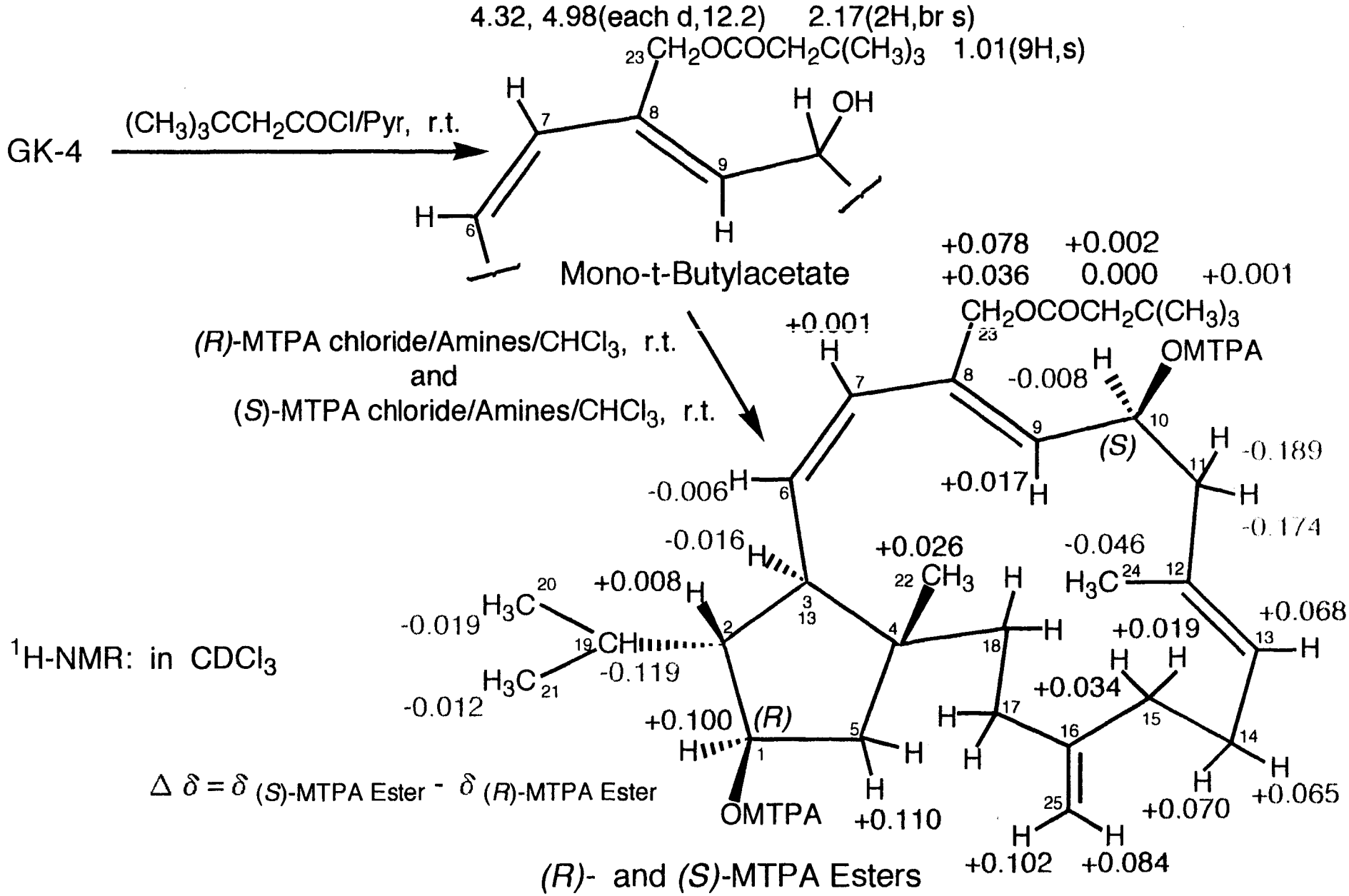
: HMBC

, : NOEDF

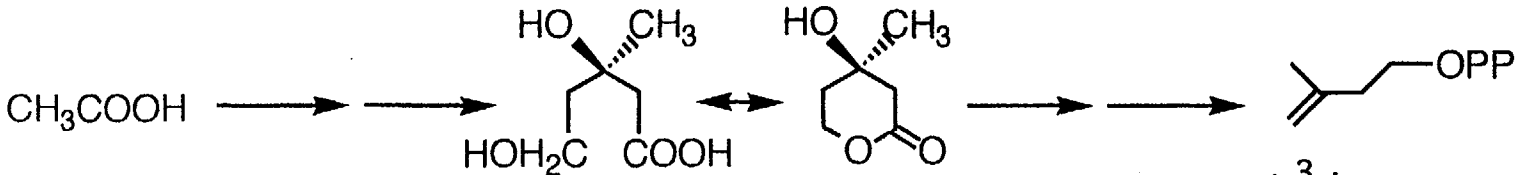
Acetylation and Benzoylation of GK-4



Stereostructure of GK-4

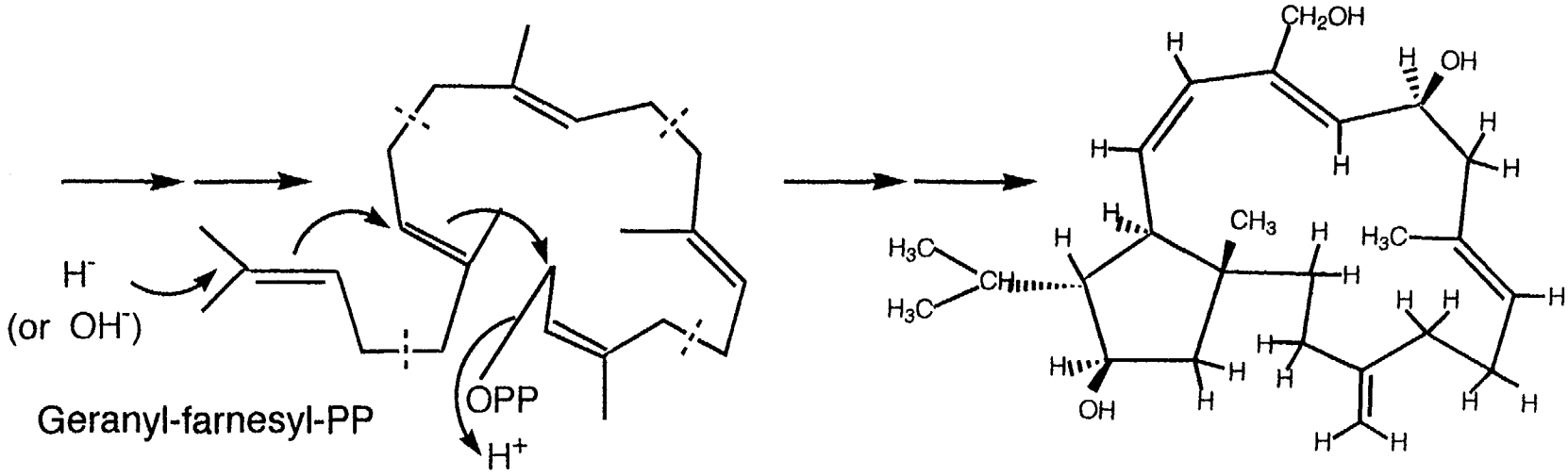


Supposed Biosynthetic Pathway for GK-4



Mevalonic acid

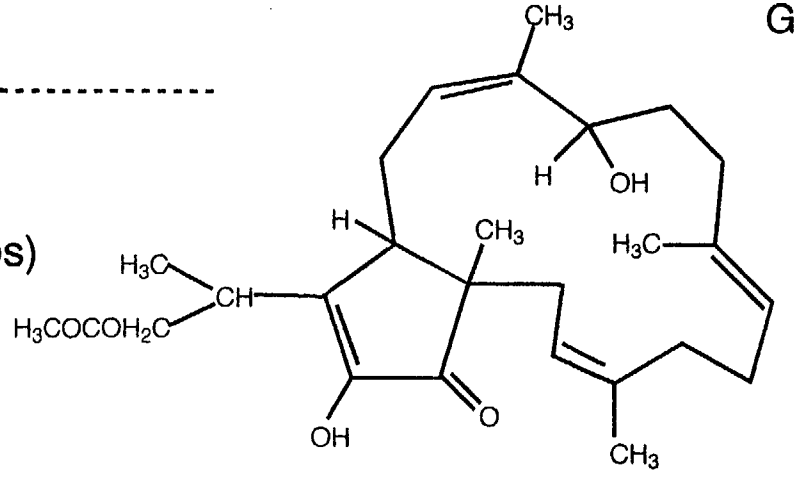
Δ3-Isopentenyl-PP



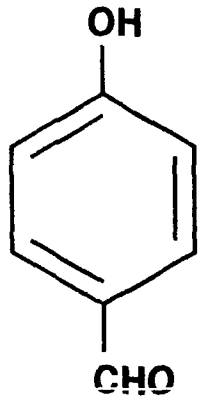
Geranyl-farnesyl-PP

GK-4 (a new sesterterpene)

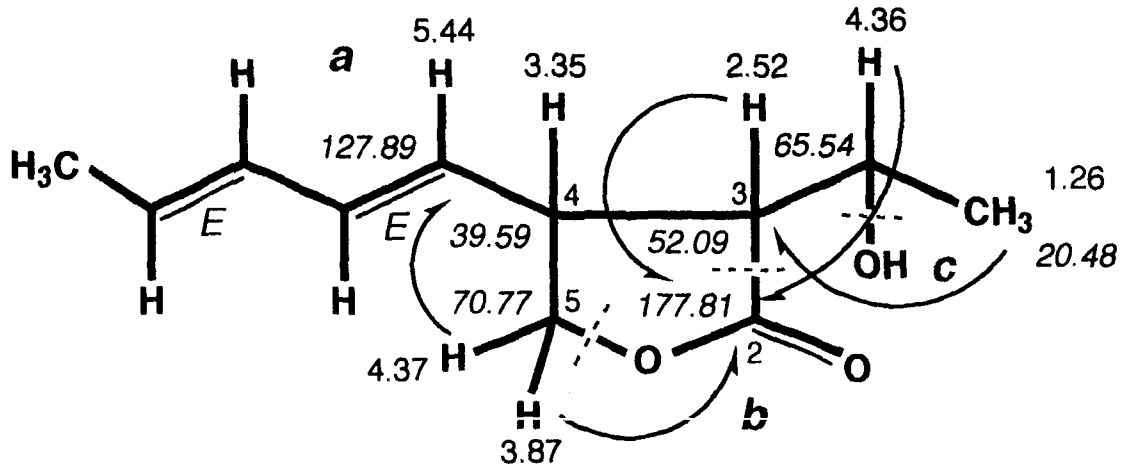
Proliferin
from *Fusarium proliferatum*
(lethally toxic to brine shrimps)
(1993)



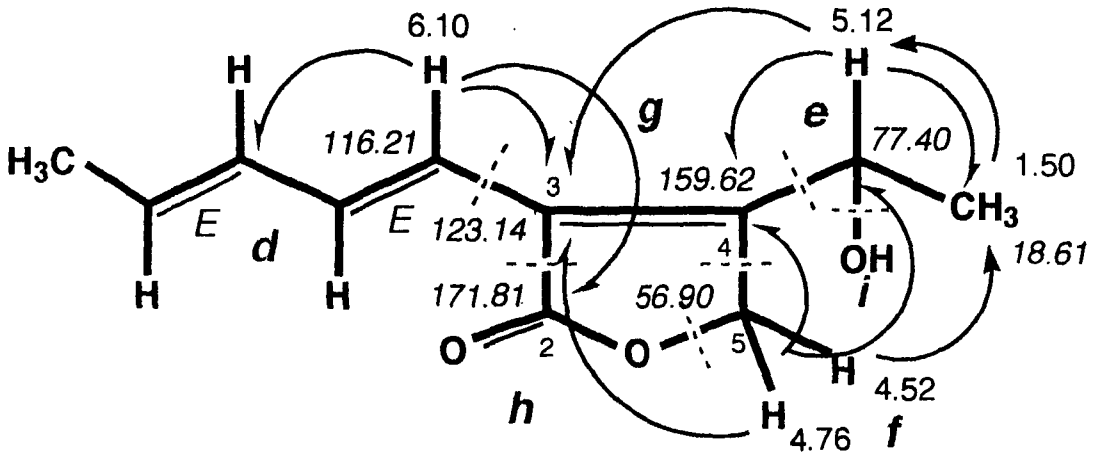
Structures of GK-1, -2, -3, and -5



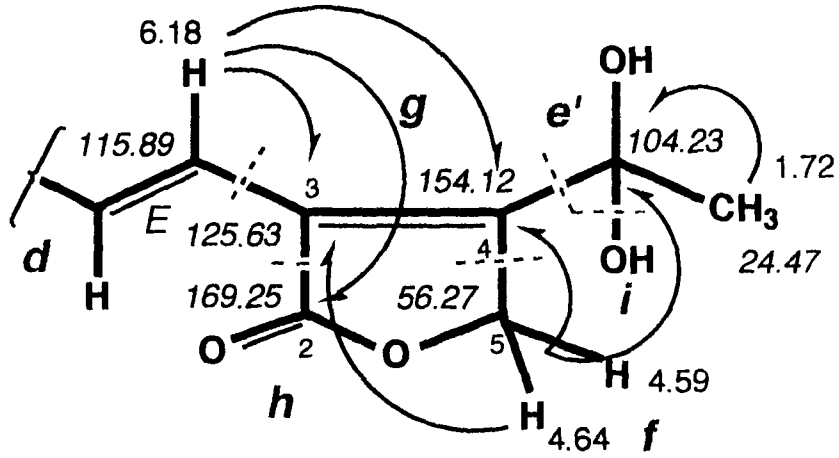
p-hydroxybenzaldehyde (GK-1)



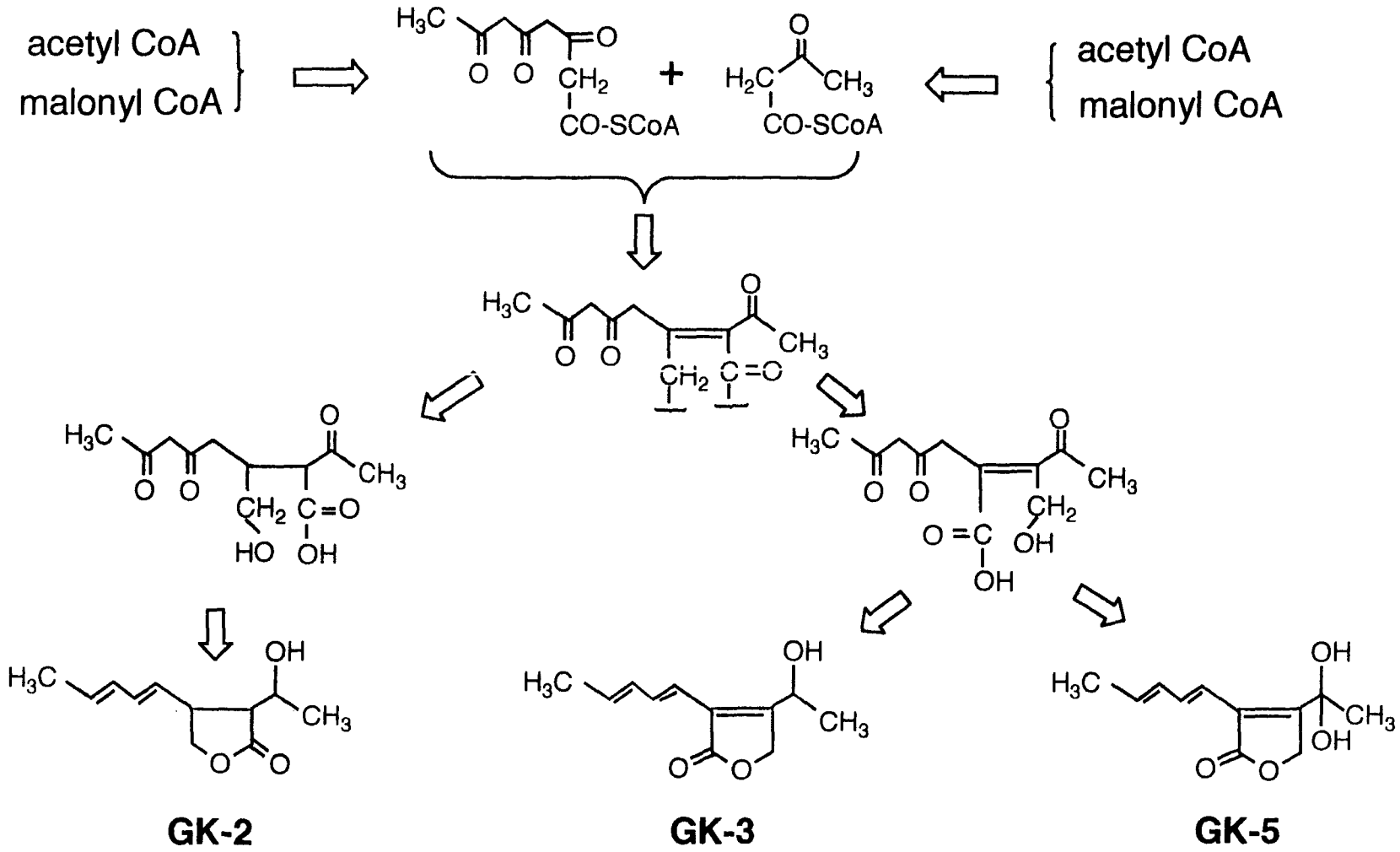
kobifuranone A (GK-2)



kobifuranone B (GK-3)

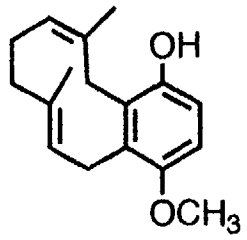


kobifuranone C (GK-5)



Biogenetic Pathway for GK-2, -3, and -5

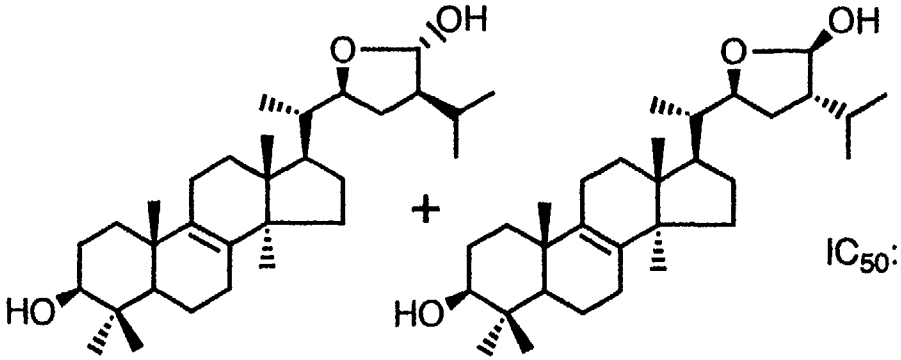
Some Immunosuppressive Components Isolated from Fungi in Our Laboratory (1)



flavidulol A

IC_{50}=8.9 μg/ml

from *Lactarius flavidulus*[1)]

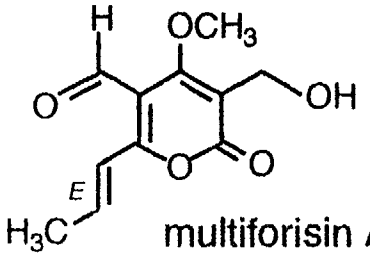


(22*S*,24*R*)-24-methyllanosta-8-en-22,28-epoxy-3β,28α-diol and
(22S,24S)-24-methyllanosta-8-en-22,28-epoxy-3β,28β-diol

IC_{50}=2.3 μg/ml

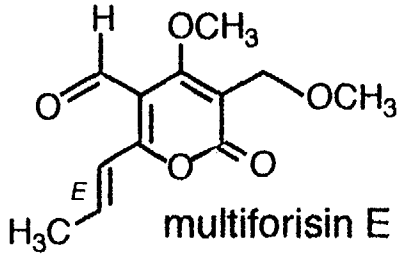
from *Pisolithus tinctorius*[2)]

IC_{50}: against Con A-induced proliferation



multiforisin A

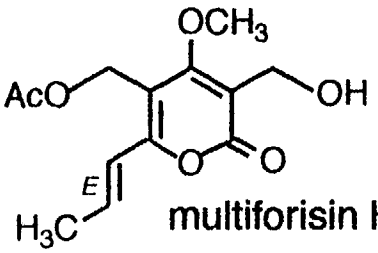
IC_{50}=0.6 μg/ml



multiforisin E

IC_{50}=5.0 μg/ml

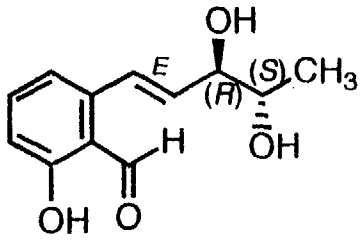
from *Gelasinospora multiforis*[3)]



multiforisin H

IC_{50}=1.8 μg/ml

from *Gelasinospora heterospora, G.longispora, G..multiforis*[4)]



sordarial

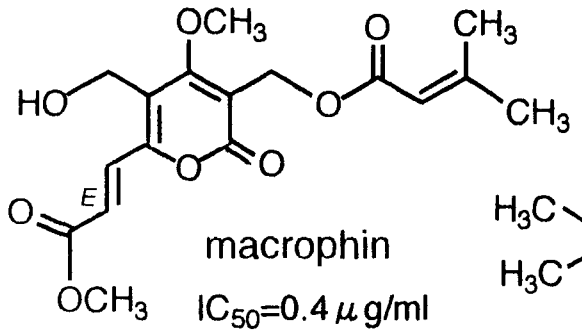
IC_{50}=6.5 μg/ml

from *Gelasinospora heterospora, G.longispora*[4)]

1) H.Fujimoto, Y.Nakayama, *et al.*, *Chem.Pharm.Bull.*, 41, 654-658 (1993)
2) H.Fujimoto, M.Nakayama, *et al.*, *Chem.Pharm.Bull.*, 42, 694-697 (1994)
3) H.Fujimoto, Y.Satoh, *et al.*, *Chem.Pharm.Bull.*, 43, 547-552 (1995)
4) H.Fujimoto, M.Sumino, *et al.*, *Chem.Pharm.Bull.*, 47, 71-76 (1999)

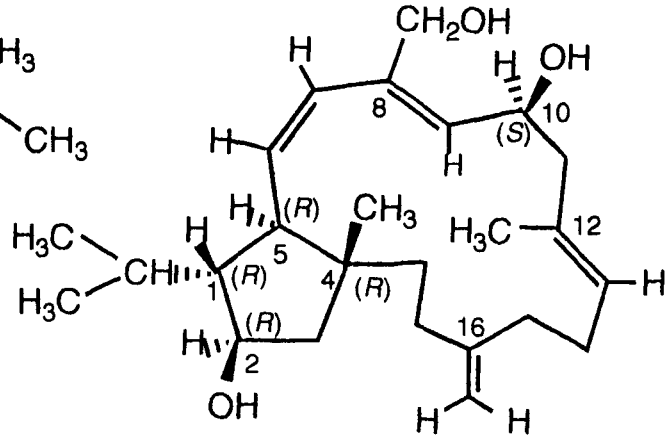
: new compound

Some Immunosuppressive Components Isolated from Fungi in Our Laboratory (2)



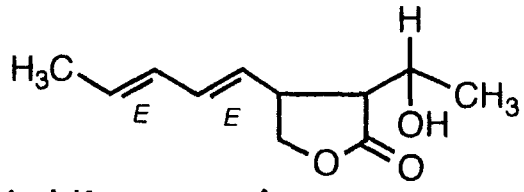
macrophin

IC_{50}=0.4 μg/ml



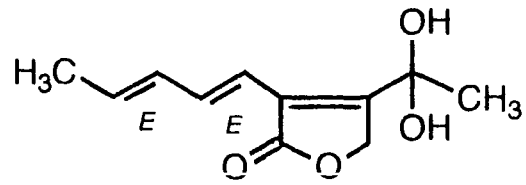
kobiin

IC_{50}=7.0 μg/ml



kobifuranone A

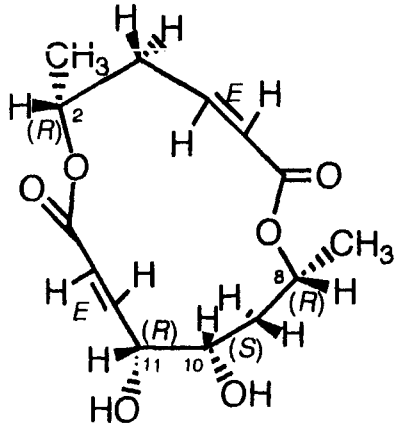
IC_{50}=39 μg/ml



kobifuranone C

IC_{50}=13 μg/ml

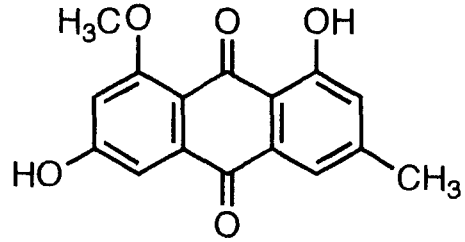
from *Gelasinospora kobi*[2]



10-*epi*-colletodiol

IC_{50}=5.0 μg/ml

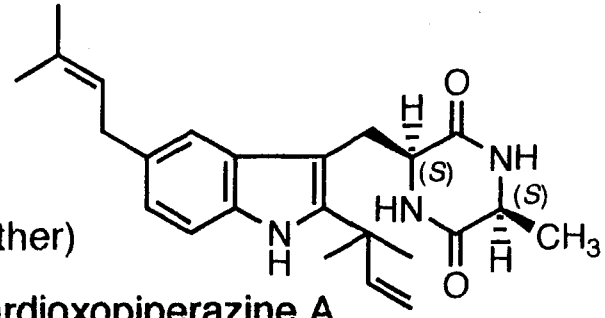
from *Diplogelasinospora grovesii*[1]



questin (emodin 8-*O*-methylether)

IC_{50}=0.3 μg/ml

IC_{50}: against Con A-induced proliferation



tardioxopiperazine A

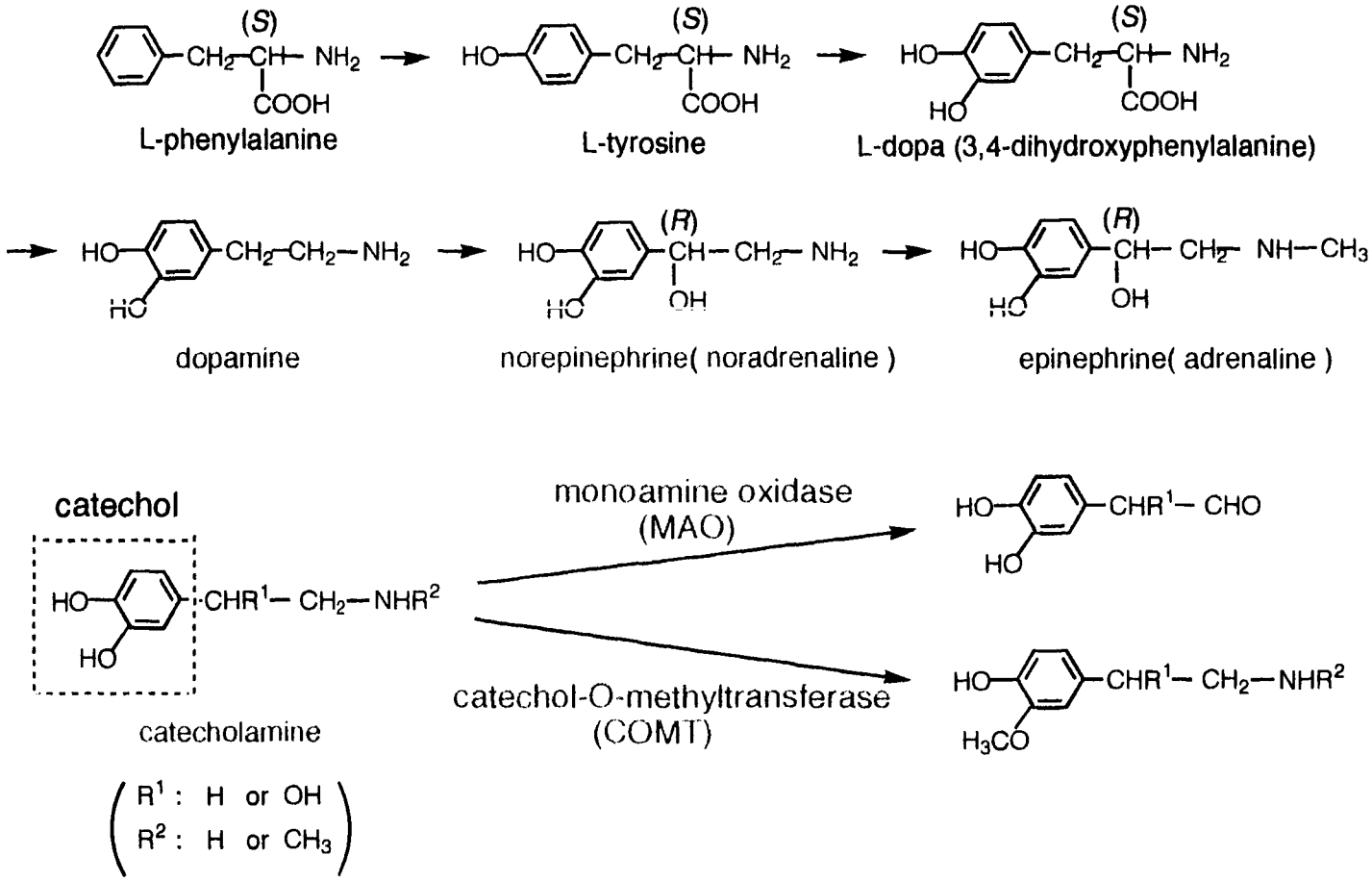
from *Microascus tardifaciens*[3]

IC_{50}=4.5 μg/ml

1) H.Fujimoto, J.Nagano, *et al.*, *Chem.Pharm.Bull.*, 46, 423-429 (1998)
2) H.Fujimoto, Y.Satoh, *et al.*, *Chem.Pharm.Bull.*, 46, 211-216 (1998)
3) H.Fujimoto, T.Fujimaki, *et al.*, *Chem.Pharm.Bull.*, 47, 1426-1432 (1999)

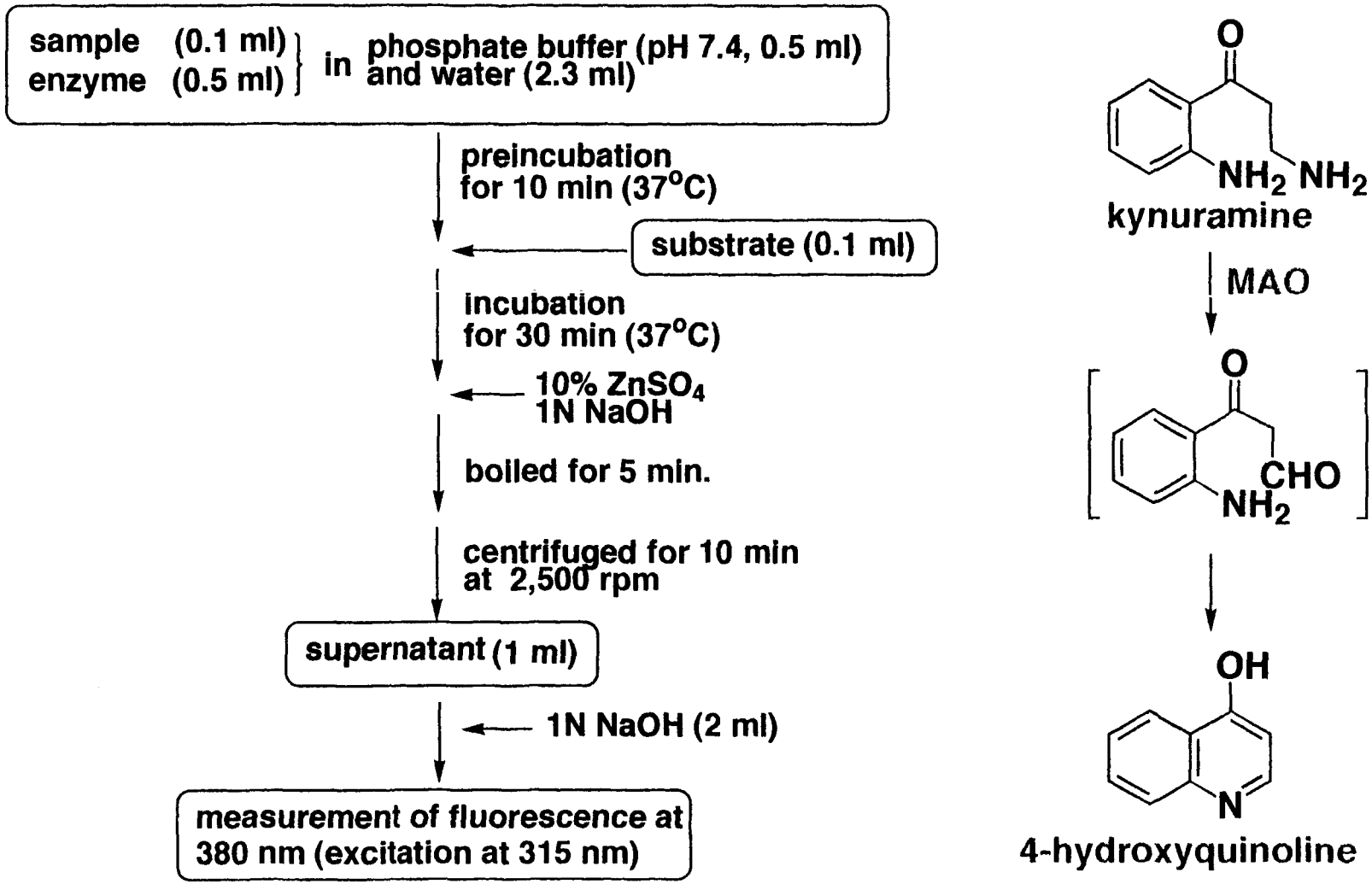
: new compound

Metabolism of Catecholamines

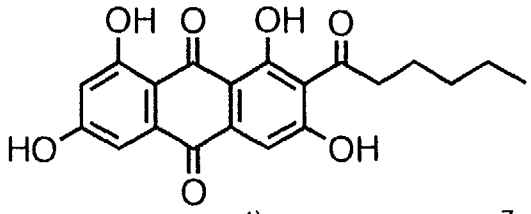


catecholamines (dopamine, norepinephrine, epinephrine): **neurotransmitter**

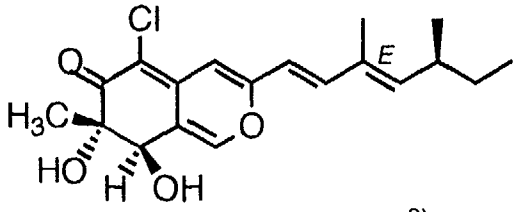
Modified Kraml's Method



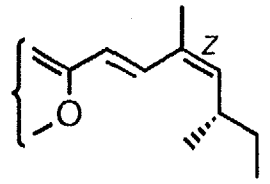
Some MAO-Inhibitory Components Isolated from Fungi in Our Laboratory



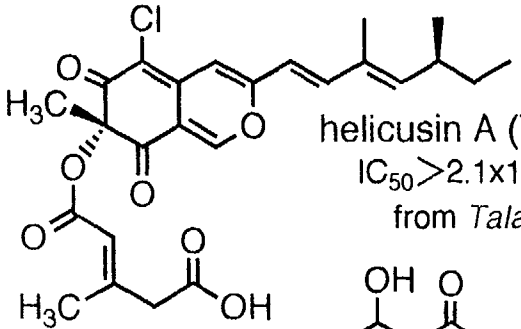
norsolorinic acid[1] IC_{50} =3.0x10^{-7}M
from *Emericella navahoensis*



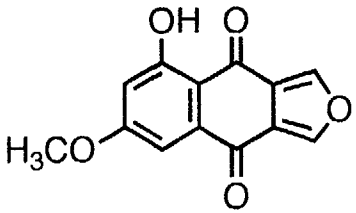
luteusin A (TL-1)[2] IC_{50} =6.6x10^{-6}M

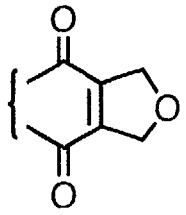


luteusin B (TL-2)[2] IC_{50} =1.1x10^{-5}M
from *Talaromyces luteus*

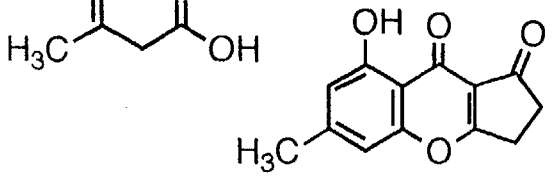


helicusin A (TH-1)[3]
IC_{50}>2.1x10^{-4}M(1.0x10^{-4}g/ml)
from *Talaromyces helicus*

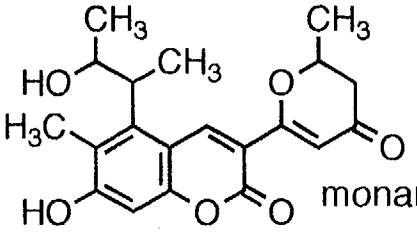




GP-A[4] IC_{50} =2.7x10^{-6}M
GP-B[4] IC_{50} =2.0x10^{-6}M
from Mycelia Sterilia of *Gelasinospora pseudoreticulata*



coniochaetone A[5] IC_{50} =2.9x10^{-5}M
from *Coniochaeta tetraspora*



monankarin A[6] IC_{50} =1.6x10^{-5}M
from *Monascus anka*

1) M.Yamazaki *et al.*, *Chem.Pharm.Bull.*, **36**, 670 (1988)
2) Y.Satoh *et al.*, *Chem.Pharm.Bull.*, **37**, 206 (1989); H.Fujimoto *et al.*, *Heterocycles*, **30**, 607 (1990)
3) E.Yoshida *et al.*, *Chem.Pharm.Bull*, **43**, 1307 (1995)
4) H.Fujimoto *et al.*, *Mycotoxins*, **41**, 61 (1995)
5) H.Fujimoto *et al.*, *Chem.Pharm.Bull.*, **44**, 1090 (1996)
6) C.F.Hossain *et al.*, *Chem.Pharm.Bull.*, **44**, 1535 (1996)

PRACTICAL TRAINING

BIOACTIVITY SCREENING

ANTIOXIDANT ANALYSIS OF HERBAL MEDICINES

August 15, 2000

Dr Ponthip Wirachwong & Varaporn Burananon

Research and Development Institute

Government Pharmaceutical Organization

Bangkok, Thailand

1. What is a free radical?
   1.1 Definition
   1.2 Hydroxyl radical
   1.3 Formation of oxygen radicals *in vivo*
   1.4 Reactive oxygen species
   1.5 Transition metal ions and free radical reaction
2. Mechanism of cell damage
3. How to characterize as an antioxidant
4. Factors affecting antioxidant status
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   5.2 A microplate assay of antioxidant capacity using 2, 7-dichlorofluorescein-diacetate in hepatoma cells
6. References

Introduction

Free radicals and reactive oxygen species (ROS) are deliberately generated in our body possibly to serve important functions. For example, activated phagocytes use ROS as a killing mechanism against invading bacteria. Although the production of free radicals or ROS seems to be useful, the excess amount of them causes damage to cells. The term 'oxidative stress' is used to describe the conditions in which the excessive production of free radical and/or ROS or the weakening of the antioxidant system occur. Because free radicals and ROS are extremely reactive, they can be very damaging to cells. Free radicals and ROS attack nonradicals and initiate chain reaction such as lipid peroxidation in cell membrane. They also damage other important molecules including proteins, carbohydrates, and DNA. It is believed that the oxidative stress play an important role in the development of aging, diseases, and cancer. Thus, the prevention of oxidative stress may be a key role for the treatment of these diseases.

In order to defend against damage from oxidative stress, living organisms develop powerful and complex antioxidant systems. Antioxidant usually acts by removing or inactivating chemical intermediates that produce free radicals. They are either produced in the body (endogenous) or derived from the diet. A large number of phytochemicals contain antioxidants, therefore, these dietary antioxidants may be very important for good health and prevention of diseases.

1 What is a free radical?

1.1 Definition

Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of 2 electrons, spinning in opposite direction. Free radicals are chemical species which have one or more unpaired electrons. Electrons are more stable when paired together in orbitals, therefore radicals are generally more reactive than nonradical species. Radicals can react with other molecules in a number of ways to combine their unpaired electrons (symbolised by •) and form a covalent bond sharing one pair of electrons. A reducing radical donates its unpaired electron to another molecule while an oxidising radical takes an electron from another molecule in order to pair. Thus, a feature of the reactions of free radicals with nonradicals is

that they tend to proceed as chain reactions. These chain reactions will be terminated when 2 free radicals meet (eq. 1).[1,2]

$$A\bullet + A\bullet \rightarrow A \qquad \text{(Eq. 1)}$$

1.2 Hydroxyl radical

Hydroxyl radical (OH•) is the most reactive free radical, whose formation plays a role in the damage caused by the exposure of tissue to high-energy radiation. When hydroxyl radical attacks to DNA, the chain reactions are initiated, leading to strand breakage, deoxyribose fragmentation and extensive chemical alterations of the purine and pyrimidine base. Imperfect repair of DNA damage induced by OH• can cause the proto-oncogene to be activated. Importantly, lipid peroxidation, which is a biological chain reaction, occur when OH• attacks the fatty acid side-chains of the membrane phospholipids. It preferentially attacks side-chain derived from fatty acid containing several double bonds, such as arachidonic acid.

$$C\text{-}H + \bullet OH \rightarrow C\bullet + H_2O \qquad \text{(Eq. 2)}$$

$$C\bullet + O_2 \rightarrow C\text{-}O_2\bullet \qquad \text{(Eq. 3)}$$

$$C\text{-}O_2\bullet + C\text{-}H \rightarrow C\text{-}O_2H + C\bullet \qquad \text{(Eq. 4)}$$

Peroxyl radical — Lipid hydroperoxide

Accumulation of lipid hydroperoxides in a membrane disturbs its function and can make it to collapse. The decomposition of lipid hydroperoxides yields a wide range of highly cytotoxic products such as aldehydes. In addition, the reactive aldehydes and peroxyl radicals can cause severe damage to membrane proteins, receptors and membrane-bound enzymes.[1,2]

1.3 Formation of oxygen radicals *in vivo*

$O_2^{\bullet-}$ radical can be generated *in vivo* in number of ways. When mitochondria are functioning, some of the electrons passing through the respiratory chain leak from the electron carriers and pass directly onto oxygen, reducing it to $O_2^{\bullet-}$. The transferring of an electron from adrenaline onto O_2 also generates $O_2^{\bullet-}$. In addition, the side effects of hyperglycaemia in diabetic patients are likely to be resulted from the oxygen radical generating from enzymatic glycosylation of proteins. Although

some of the $O_2^{\bullet-}$ production *in vivo* may be accidental, much is functional. Thus, the formation of $O_2^{\bullet-}$ occur when the phagocytes (such as monocytes, neutrophils, eosinophils and most types of macrophage) are activated. The superoxide radical is generated from the 1-electron reduction of oxygen (eq. 5). It has been found that the superoxide dismutase (SOD) in all human cells can remove superoxide radical by catalysing a dismutation reaction to O_2 and H_2O_2 (eq. 6).

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad \text{(Eq. 5)}$$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(Eq. 6)}$$

Interestingly, neutrophils use H_2O_2 produced from the dismutation of $O_2^{\bullet-}$ to oxidise chloride ions to hypochlorous acid, a powerful antibacterial agent, as another killing mechanism.[1,2]

1.4 Reactive oxygen species

The term 'reactive oxygen species' is used to describe free radical ($O_2^{\bullet-}$, OH•) or nonradical (such as H_2O_2, HOCl, singlet oxygen). According to Fig.1, reactive oxygen species generated in the cells cause the membrane alteration, macromolecule oxidation, loss of ionic equilibration and lesion to nucleic acids.[1,2]

1.5 Transition metal ions and free radical reaction

Most transition metals have variable oxidation numbers, e.g. Fe^{++}, Fe^{+++}, Cu^+, and Cu^{++}. Thus, most transition metal ions are remarkably good promoters of free radical reactions.

$$Fe^{+++} + e^- \longleftrightarrow Fe^{++} \quad \text{(Eq. 7)}$$

$$Cu^{++} + e^- \longleftrightarrow Cu^+ \quad \text{(Eq. 8)}$$

Iron and copper ions can accelerate the reaction of lipid peroxidation. The transition ions are involved in the formation of the initiating species or species which are able to abstract hydrogen. Moreover, they can decompose lipid hydroperoxides into peroxyl and alkoxyl radicals that can abstract hydrogen and accelerate the chain reaction of lipid peroxidation. This is represented in the equations below, in which $L^{\bullet}$ symbolises a carbon-centered radical.

$LOOH - Fe^{++}(Cu^{+}) \rightarrow LO^{\bullet} - Fe^{+++}(Cu^{++}) - OH^{-}$ (Eq. 9)

Alkoxyl radical

$LOOH - Fe^{+++} \rightarrow LOO^{\bullet} - Fe^{++}(Cu^{+}) - H^{+}$ (Eq. 10)

Peroxyl radical

$LO^{\bullet} + LH \rightarrow LOH + L^{\bullet}$ (Eq. 11)

$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (Eq. 12)

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (Eq. 13)

The end-products of these reactions include the cytotoxic aldehydes mentioned previously, as well as hydrocarbon gases such as ethane and pentane.[1,2]

2 *Mechanism of cell damage*

Oxidative stress in cells and tissues results from increased generation of reactive oxygen species and/or from decreases in antioxidant defenses. The increase of $O_2^{\bullet-}$, H_2O_2 production can occur as a result of:

1. the increase of O_2 concentrations
2. the addition of toxins which increase intracellular formation of reactive oxygen species such as alloxan, paraquat or adriamycin
3. the activation of phagocytes at a localized site for the killing of bacteria.

The oxidative stress results in the damage of DNA, increase of intracellular free iron and increase of lipid peroxidation which are subsequently leading to cell death as shown in the Fig. 2.[1,2]

3 *How to characterise as an antioxidant*

According to the summary reported by Halliwell (1990), there are 6 questions to ask whether the compounds to be considered as antioxidant *in vivo*.

1) What biomolecule is the compound supposed to protect? For example, an inhibitor of lipid peroxidation is unlikely to be useful if the oxidative damage is mediated by an attack upon proteins or DNA.
2) Is the compound present *in vivo* at or near that biomolecule at sufficient concentration? For example, many compounds have been suggested to act as OH• scavengers *in vivo*. In order to compete with biological molecules for

OH• a scavenger has to be present at at least millimolar concentrations *in vivo*. Most drugs never achieve this sort of concentration.

3) How will the compound protect? by scavenging ROS. by preventing their formation or by repairing damage?
4) For naturally occurring antioxidants, is antioxidant protection the primary biological role of the molecule or a secondary one? For example, SOD has probably evolved as an antioxidant enzyme. By contrast, transferrin has probably evolved as an iron transport protein, although the binding of iron ions to transferrin stops them accelerating radical reactions, giving this protein an important secondary role in extracellular antioxidant defense.
5) If the antioxidant acts by scavenging a ROS, can the antioxidant-derived radicals themselves do biological damage?
6) Can the antioxidant cause damage in biological systems different from those in which it exerts protection?

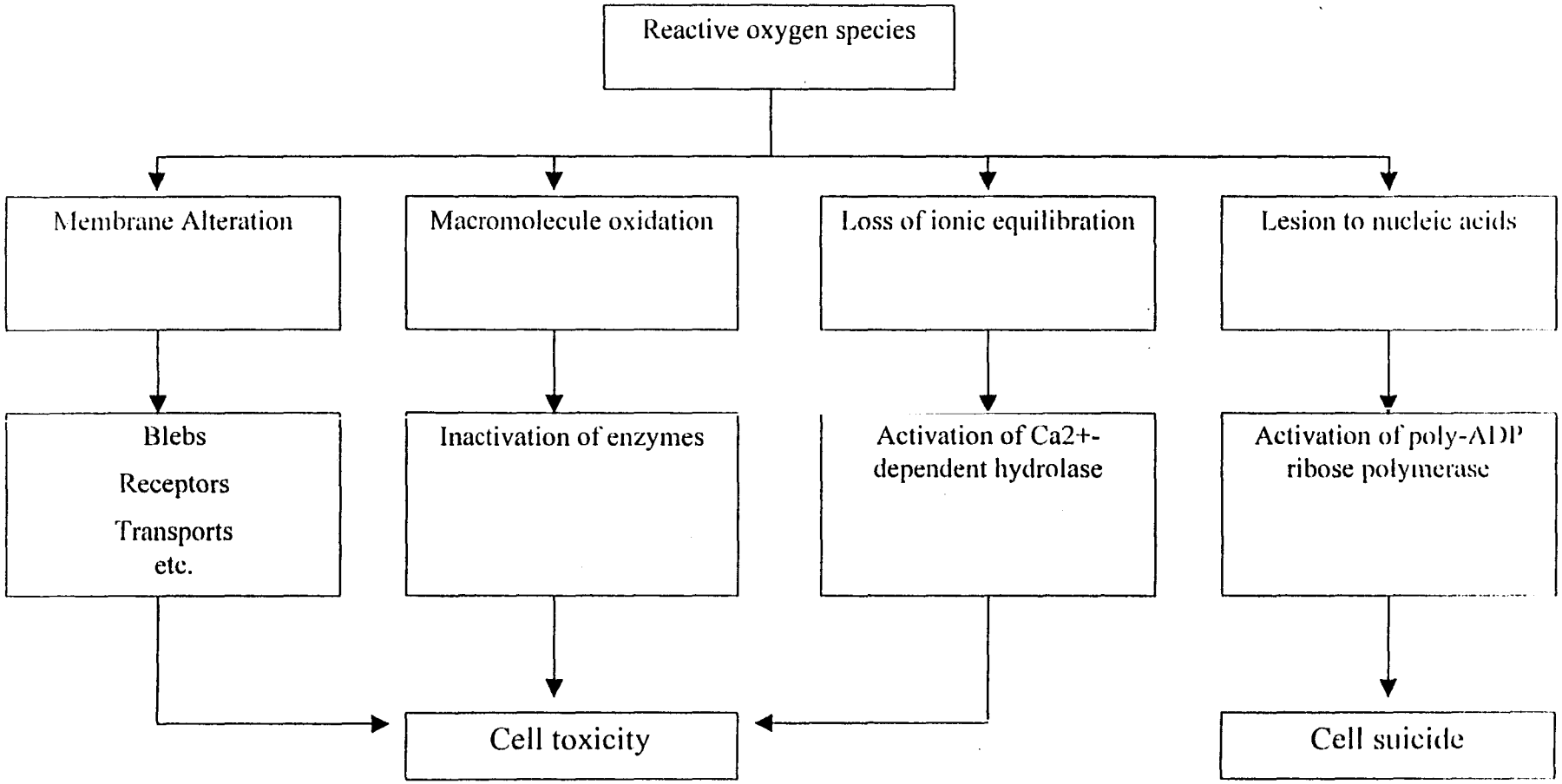


Fig. 1. Schematic illustrating the multiple damages caused by reactive oxygen species.[1]

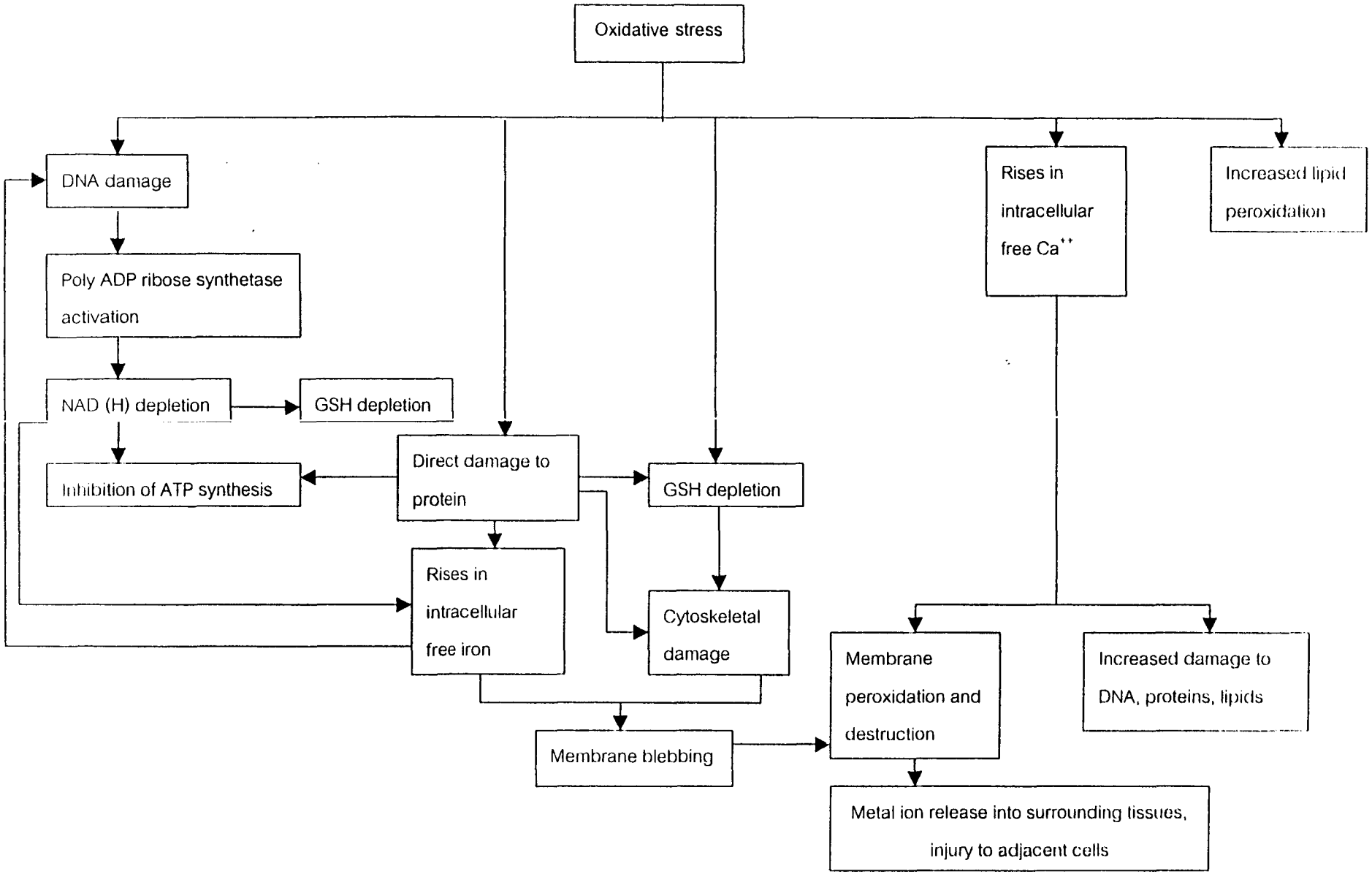


Fig. 2. Schematic illustrating the multiple damages caused by oxidative stress [2]

4 Factors affecting antioxidant status

The antioxidant status is affected either from endogenous production or from increased dietary supply. It is also affected by the production of free radicals and ROS, which cause increased utilization of antioxidants. There are major factors affecting the antioxidant status including genetic factors, diet, environment (pollutants, tobacco smoke, UV radiation), alcohol, injury, diseases, and physiological status.[1,2]

5 ***Methods for evaluation of antioxidant status***

5.1 Analysis of antioxidant capacity by the reaction of 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS)/ hydrogen peroxide/ metmyoglobin

Method which has been widely developed, is based on the inhibition of the accumulation of oxidised products. The formation of free radical species is inhibited by the addition of antioxidants which exert free radical scavenging activity. Thus, the free radical that is generated, is used as an end point measured from the reactions. The present method which has been developed by Miller *et al.* (1993; 1996), is based on a decoloration reaction. The reaction is initiated by adding ABTS and hydrogen peroxide to produce ABTS radical. The generation of ABTS radical is allowed to proceed until a stable color of ABTS radical occurs. Antioxidants which have scavenging activity are, therefore, decolor a mixture of ABTS radical, giving an index of antioxidant capacity.[3,4,5,6]

Materials and methods

Myoglobin, Potassium ferricyanide, 2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma. Sephacryl S-100 HR was obtained from Pharmacia biotech. Hydrogen peroxide (H_2O_2), sodium chloride (NaCl), Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were obtained from Merck.

Preparation of sephacryl S-100 HR column

After assembling the column, the column was mounted vertically on a laboratory stand. The column was flushed with phosphate buffer saline (PBS), pH 7.4.

leaving buffer 2/3 of the column. The running buffer was set to flow at 1.9 ml/min while packing the gel. The gel slurry was prepared by adding 12 ml of sephacryl S-100 HR suspension with 4 ml of PBS and was poured into the column. To prevent the introduction of air bubbles, the gel slurry was poured down a glass rod held against the wall of the column. Finally, the gel was packed until the surface is approximately 10 mm below the end of the glass tube. The packed column was equilibrated with 50 ml PBS at 0.1 ml/min.

Purification of metmyoglobin

Myoglobin (400 μM) which was dissolved in PBS, pH 7.4, was added to an equal volume of freshly prepared 740 μM potassium ferricyanide. To purify metmyoglobin, the mixture was loaded into a prepared sephacyl S-100 HR column equilibrated in the buffer. The brown solution of metmyoglobin fraction was collected. The final concentration of purified metmyoglobin was calculated by applying the Whitburn equations:

$$[\text{Metmb}] = 146\,A490 - 108\,A560 + 2.1\,A580$$
$$[\text{Ferryl Mb}] = -62\,A490 + 242\,A560 - 123\,A580$$
$$[MbO_2] = 2.8\,A490 - 127\,A560 + 153\,A580$$

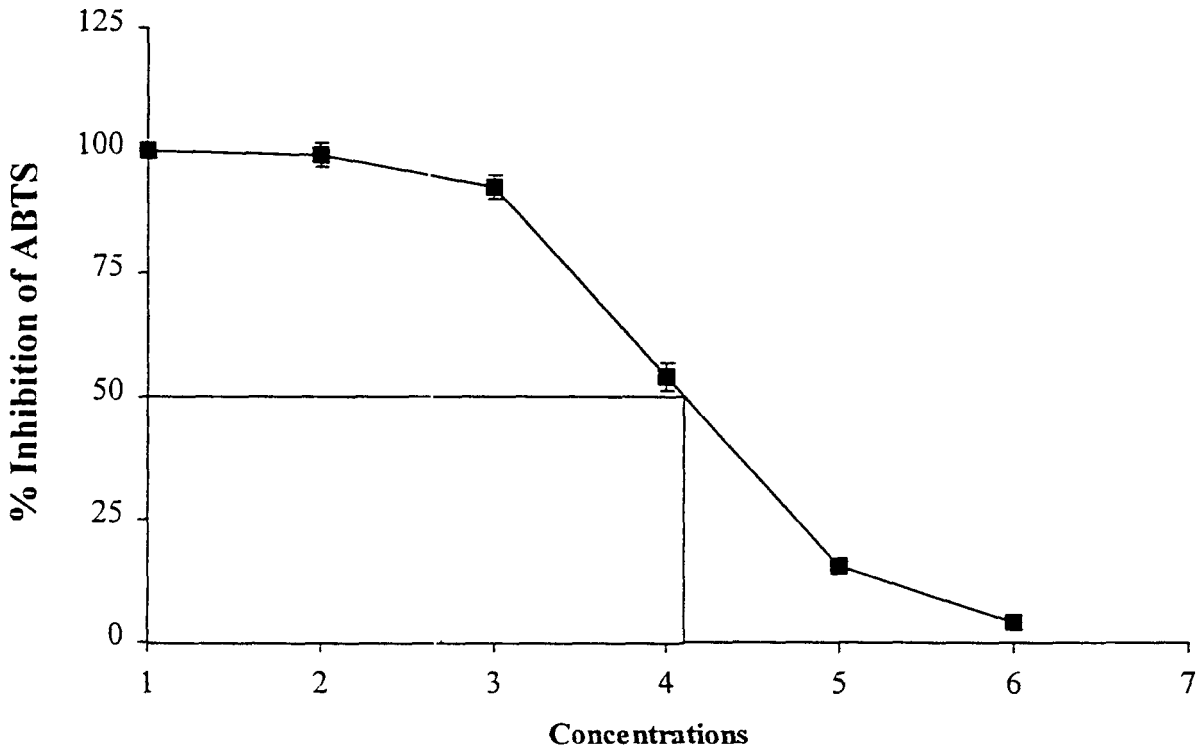
Where Mb is myoglobin. These equations are derived by solving simultaneous equations based on Beer's law, measuring the absorbance at 490, 560 and 580 nm, and substraction the reading at 700 nm to correct for background absorbance. The purity of the metmyoglobin was estimated by applying all three equations. Normally, the metmyoglobin fraction is > 95% of the total haem protein. After purification of metmyoglobin, the column was re-equilibrate with 50 ml PBS at 0.1 ml/min to make it ready for the subsequent use.

Antioxidant analysis

ABTS (300 μl, 500 μM), 36 μl of 70 μM metmyoglobin and 477 μl of buffer were mixed and the reaction was initiated by addition 167 μl of 450 μM hydrogen peroxide. The final concentrations of metmyoglobin, ABTS and hydrogen peroxide were 2.5 μM, 150 μM and 75 μM, respectively. The mixture was held for 1 hour and

then 20 µl of test sample was added. The absorbance was read at 750 nm. A curve of ABTS radical cation (%) against sample concentration was constructed and the IC_{50}, a concentration required to produce 50% of the maximal inhibition response, was determined from this concentration response curve.

$$\% \text{ inhibition of } ABTS^{\bullet+} = \frac{\text{mean of } OD_{750sample}}{\text{mean of } OD_{750control}} \times 100$$



5.2 A microplate assay of antioxidant capacity using 2′,7′-dichlorofluorescein-diacetate in hepatoma cells

This method is based on the fluorimetric assay which employs a fluorescent probe, 2′,7′-dichlorofluorescin-diacetate (DCFH-DA) to measure reactive oxygen species (ROS) formed within the cells. The measurement of oxidative product formation within cells is based on the assumption that, due to its polarity, fluorescent DCF remains intracellular. This, however, does not exclude the formation of extracellular ROS which might cause the oxidation of DCFH-DA to its fluorescent

counterpart in supernatants. The present assay was slightly modified from those reported by Yang, *et al.* (1999) and Rosenkranz *et al.* (1992). DCFH-DA, a non-polar compound, diffuses through the cell membrane and is enzymatically hydrolysed by intracellular esterases to nonfluorescent derivative, 2',7'-dichlorofluorescin. This nonfluorescent derivative is polar and trapped within the cells. It is then oxidised to highly fluorescent 2',7'-dichlorofluorescin (DCF) in the presence of intracellular ROS. Thus, the DCF fluorescent intensity is considered to be parallel to the amount of ROS formed intracellularly.[7,8]

Materials

Cell culture media (MEM), fetal bovine serum and other medium supplements were obtained from Gibco. DCFH-DA was obtained from Sigma. NaCl, hydrogen peroxide were obtained from Merck. Tissue culture plastics were obtained from Nunc.

Cell culture

The human hepatoma cell line (HepG2) was purchased from American Type Culture Collection. The cells were cultured in MEM supplemented with 10%FBS, 1mM pyruvate and 0.1mM nonessential amino acid. They were seeded at 7×10^6 cells per 250 ml flask and grown up to confluency in 7 days in incubator set at 37°C and 5% CO_2. At confluency, cells were washed twice with PBS and trypsinized with 0.25% trypsin in 1:5000 EDTA solution for 8-10 minutes. Cells were counted using haemocytometer after FBS-containing medium was added. The cells were seeded at the density of 7×10^6 cells in 250 ml flask and subsequently kept in culture or used in the assay for antioxidant capacity. Before starting the assay, the cells were centrifuged for 2×3 minutes at 200 rpm and diluted to 1×10^6 cells/ml of iced-cold PBS.

Assay for antioxidant capacity by measuring of intracellular ROS

The assay is carried out as follows. Firstly, the 50µl of 1×10^6cells/ml hepG2 was added into 96-well plate. Before adding 1µg/ml DCFH-DA, hydrogen peroxide was added at a final concentrations of 0.25 mM. The microplate was incubated at 37° C for 60 minutes after antioxidant was added. DCF fluorescence intensity was detected using a luminescence spectrometer (Perkin-Elmer LS-50B) connected to plate reader accessories with excitation and emission wavelength at 485 and 530 nm.

respectively. A curve of intensity unit (%) against sample concentration was constructed and the IC_{50}, a concentration required to produce 50% of the maximal inhibition response, was determined from this concentration response curve as shown previously.

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GOOD LABORATORY PRACTICE OF HERBAL MEDICINAL PRODUCTS

Dr. Krisana Kraisintu

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname : Kraisintu Other name : Krisana

Title (Mr, Mrs, Dr, Professor, etc) Dr.

Current Position : Head of Research and Development Institute

Current Place of Work : Government Pharmaceutical Organization

Educational profile:

Year	Place of Study	Qualification	Field of study
1975	Chiangmai University	Bachelor	Pharmaceutical Sciences
1978	Strathelyde University	M. Sc.	Pharmaceutical Analysis
1981	Bath University	Ph. D	Pharmaceutical Chemistry

Professional Experiences :

1995 Executive Director of Thai Herbal Products Company

Current interests :

Requirements for raw plant materials

While a regulatory system for raw plant materials used in individual dispensing would be difficult and impractical to implement, plant materials identified as toxic should be subjected to specific regulatory procedures. Plant materials classified as toxic should be dispensed only by appropriately qualified practitioners.

At all levels of handling of raw plant materials, clear and accurate identification and labelling is paramount. Countries should also give consideration to mechanisms for controlling contamination of raw plant materials with pests, microorganisms, aflatoxins and other mycotoxins, pesticides, heavy metals and other foreign matters.

Requirements for processed plant materials

1 Processed plant materials may be supplied as ingredients to practitioners or as starting materials to product manufacturers. In these cases, the following information should be supplied:

(a) taxonomical classification of the plant including genus, species and family;

(b) common names;

(c) expected countries of origin;

(d) part of the plant used and its condition (such as fresh aerial part; dried root and rhizome, sliced or decorticated);

(e) year, season, preliminary preparation and drying and methods of collection, if necessary;

(f) the method of preparation, including details of new processing techniques; and

(g) the excipients used (where relevant) for commercial reasons.

2 Where, for commercial reasons, the supplier/manufacturer of processed medicinal materials does not wish to provide details of the extraction methodology or excipients used to the manufacturer or practitioner, a notification (listing) or registration procedure could be implemented. In this case, particularly if using new processing methods, in addition to the information required under 8.3.1, the following information, may also be required, if relevant:

(h) characterizing compounds of the processed medicinal material and the chromatogram of the characterizing compounds;

(i) data on long-term toxicity tests, if appropriate;

(j) data on mutagenicity tests;

(k) data on carcinogenicity tests, if appropriate;

(k) data on reproductive and developmental toxicity tests when necessary;

(l) stability tests;

(m) quality standard, including the assay or limit of toxic ingredients, microorganisms, mycotoxins, heavy metals and pesticide, insecticide and herbicide residues; and

(n) reports on clinical trials, when necessary.

Requirements for medicinal herbal products

For medicinal herbal products a notification (listing) or registration procedure should be used in most cases. The manufacturers, distributors or importers should provide information on items listed below in relation to the product. In general, the requirements for medicinal herbal products would be pertaining to the product, however, data on individual components may in some circumstances be required. Efforts should be made to achieve high standards of practice in this area wherever possible.

(1) For traditionally used medicinal herbal products the following are needed:

(a) name of the product;

(b) list of ingredient(s) (active and inactive) of the product with scientific name(s), part of the plant used, and quantity; and with reference to the source text for the prescription, if available;

(c) the list of plant ingredient(s) of the product with taxonomic classification, including species, genus, and family;

(d) methods and technology used in manufacture;

(e) physical and chemical identification tests;

(f) quality standards for the ingredients when necessary (which may include the limit of residue of heavy metals and pesticides, insecticide and herbicide);

(g) quality standards for the products;

(h) stability tests;

(i) therapeutic uses and dosage;

(j) evidence of traditional use or recent clinical experience with the product in the form proposed, to support the safety and efficacy of the product;

(k) package and packaging materials; and

(l) content on label or package insert.

For those traditionally used herbal medicines with new dosage forms or new administration routes, the following additional data may be needed:

(m) comparative data on bioavailability.

For special dosage forms, such as injections and nebulisers, additional data may be required.

For those traditionally used herbal medicines with new indications, the following data, additional to items (a) to (l) may be needed:

(n) reports on clinical trials.

(2) For new medicinal herbal products which contain herbs with no traditional history of use, the following data should be submitted, in addition to the data on items (a) to (l) listed above:

(a) data on pharmacodynamic, bioavailability tests, and general pharmacological studies;

(b) data on acute toxicity tests;

(c) data on long-term toxicity tests, if necessary;

(d) data on mutagenicity tests, if necessary;

(e) data on carcinogenicity tests, if necessary;

(f) data on reproductive and developmental toxicity tests, if necessary; and

(g) reports on clinical trials.

(3) For importing countries, confirmation of the regulatory status in the country of origin should be required. Countries should consider extending the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce to cover medicinal herbal products. Where countries and areas have not yet adopted this scheme, the importers should submit a certificate of free sale and certificates of Good Manufacturing Practice (GMP) for the country of origin. Those certificates should be issued by the drug regulatory authority of the country of origin. After reviewing all the documents, a registration or notification (listing) may be given to medicinal herbal products imported by individual importers and the registration or listing number must appear on the labels of medicine.

Label requirements

It is recommended that the following is printed on the product label in the official language(s) used by the countries or areas:

(a) name of product;

(b) name and quantity (in dry weight when relevant) of active ingredient(s);

(c) dosage form;

(d) directions for use including indications, dosage, mode of administration, duration of use, age group limitations, and use during pregnancy and lactation;

(e) warning statements and relevant contraindications, adverse effects, if any, and overdose information when relevant;

(f) batch number;

(g) expiry date;

(h) storage conditions;

(i) name and address of manufacturers and/or importers; and

(j) registration or notification (listing) number.

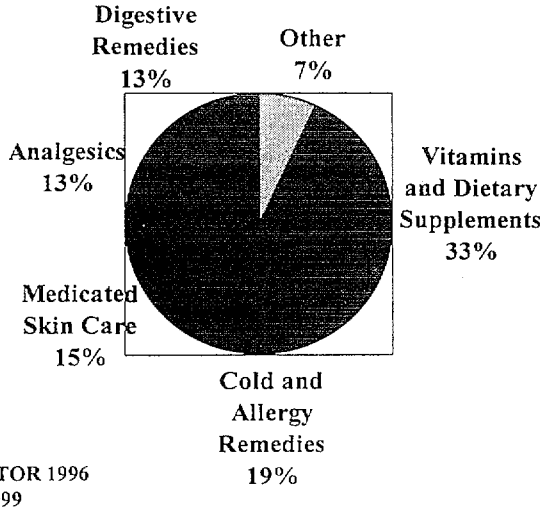
The scientific name of active ingredient(s), in addition to the common name in the language of preference of the national regulatory authority, should be used.

The label and package insert should be "user-friendly". Easy and understandable information should be provided.

The drug regulatory authority may provide to industry directions on labelling and on allowable indications and claims.

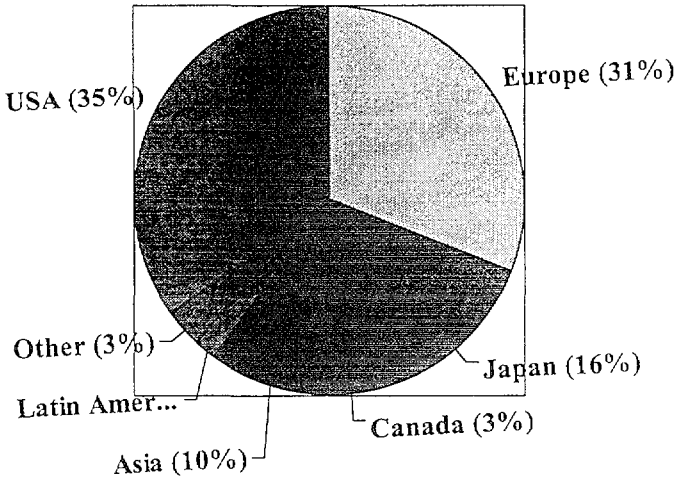
Health Care Products (OTC) - World Market

1998, % of total
Total : $75bn



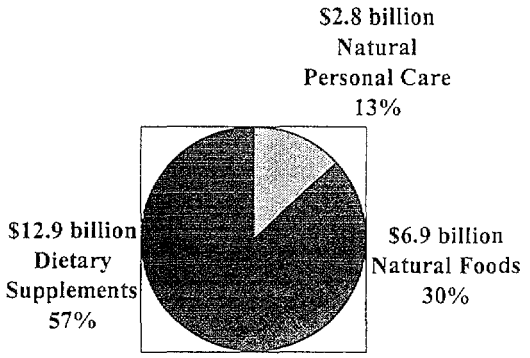
SOURCE: UROMONITOR 1996
ECONOMIST MAY 1999

The $64 Billion Global Nutrition Market



SOURCE: NUTRITION BUSINESS JOURNAL, USA MARCH 1999

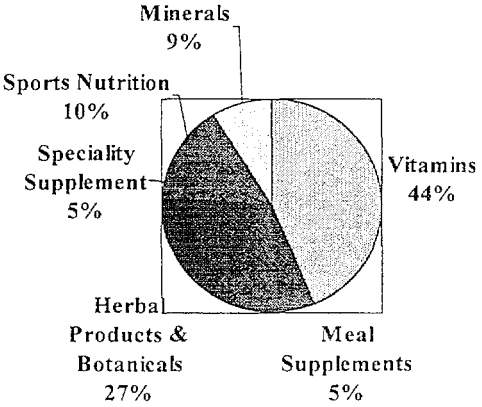
$23.2 Billion U.S. Nutrition Industry Product Segments 1997



SOURCE: NUTRITION BUSINESS JOURNAL, USA MARCH 1999

The $12.9 Billion Dietary Supplements Industry

Supplement Categories 1997



SOURCE: NUTRITION BUSINESS JOURNAL, USA MARCH 1999

Industrial Exploitation of Indigenous Medicinal and Aromatic Plants:

Quality Assessment of Phytopharmaceuticals

Dr. Krisana Kraisintu

Research and Development Institute

Government Pharmaceutical Organization

What's in the Name?

- **Herbal medicines**
- **Botanicals**
- **Phytopharmaceuticals**
- **Traditional Chinese medicines (TCM)**

What's in the Name?

- **Ayurvedics**
- **Complementary Alternative Medicine (CAM)**

Phytopharmaceuticals should always contain the ***active principles together with coexisting materials from the source plant,*** these additional materials having a greater or lesser beneficial influence upon the activity of the drug.

Major classes of natural products

- **Bio-saponins; steroids and triterpenoids**
- **Flavonoids: bioflavonoids and biflavonoids**
- **Amino acids: non-protein, biocomplexes**
- **Proteins and phytoamines**
- **Anti-oxidants**
- **Alpha hydroxy acids**
- **Formulation aids**
- **Vitamins (esp. Vit. A and E)**

Significant Consumer Interest Shift towards Natural Product

†***Consumer perceive modern health care to be ineffective, expensive, and having undesirable side effects.***

†***People today want to prolong their lives, protect their good health and promote a more active lifestyle.***

Significant Consumer Interest Shift towards Natural Product

- With more leisure, interests have turned toward health and fitness, preventive instead of cure.
- Consumers preferred "natural" products instead of synthetic "chemicals".

Phytopharmaceuticals as "Nutritional Supplements"

- Advantage
  - Easy access for consumers
  - Low cost

Phytopharmaceuticals as "Nutritional Supplements"

- Disadvantage
  - No quality control
  - No proof of safety or efficacy
  - Problems in labeling

Phytopharmaceuticals as "Drugs"

- **Research:**
  - **Evaluation and validation; methodology and outcome measurement**
- **Quality Assurance:**
  - **In process monitoring;source material**
- **Clinical Trials:**
  - **Requirement and design**

Disadvantage of traditional methods:

- Authenticity and purity of raw material not known
- Variability of raw material quality
- Post-harvest deterioration of raw material
- Seasonal nature of plants
- Non uniformity of dosage
- Poor stability of the preparation
- User difficulties owing to bulkiness and transport

Advantages of introducing modern technology:

- ✓ **Verification of authenticity and purity**
- ✓ **Control of quality of raw material**
- ✓ **Proper post-harvest treatment**
- ✓ **Controlled and efficient processing methods which are reproducible**
- ✓ **Standardized product and therefore uniformity of dosage**
- ✓ **Conversion into conveniently handled dosage forms**
- ✓ **Significant increase in stability**

Requirement for Any Phyto-pharmaceutical when for Human Use

- *Purity*
- *Constancy*
- *Stability*
- *Residual solvent*
- *Pesticides*

Parameters Influencing Quality of Plant Material and Quality of the Extracts

- **Botany**
  - *Use of the correct species*
  - *Presence of other species or varieties*
  - *Presence of contaminants (other plants or different parts of the plants)*

Parameters Influencing Quality of Plant Material and Quality of the Extracts

- **Botany**
  - *Harvesting period*
  - *Area of origin*
  - *Storage of the plant material*
  - *Microbial counting*

Parameters Influencing Quality of Plant Material and Quality of the Extracts

- Chemistry
  - *Content of active principles*
  - *Qualitative composition of the plant*
  - *Extractive content*
  - *Ratio among the various compounds*
  - *Content of heavy metals*
  - *Solvents used for extraction*

Regulatory proposals dealing with herbal medicines

A first step in the right direction

1. Harmonisation of the assessment of herbal remedies

The European Agency for the Evaluation of Medicinal Products (EMEA)

Set up criteria for the assessment of quality, safety and efficacy of herbal medicinal products

2. Regulatory proposals for revision of bibliographical applications

Assessment of appropriate efficacy and safety

- ***impurity - related substances profile***
- ***decomposition products arising during storage***

European Scientific Co-operative on Phytotherapy (ESCOP)

World Health Organisation (WHO)

Scientific monographs on herbal drugs

3. Proposals for revision of Good Manufacturing Practice

GMP

Agricultural production GAP

- *selection of seeds*
- *conditions of cultivation*
- *harvesting*

generating reproducible quality herbal drugs

4. Regulatory proposals dealing with quality of herbal medicines

GLP

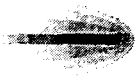
4.1 Proposals for revision of the control of starting materials

Active substance

- *Herbal drugs*
- *Herbal drug preparations*

Other constituents

- *solvents*
- *diluents*
- *preservatives*



GHP

- *site of collection*
- *time of harvesting*
- *stage of growth*
- *treatment during growth with pesticides*
- *drying*
- *storage conditions*

Herbal drugs with constituents of known therapeutic activity

Assays of their contents

Herbal drugs without constituents of known therapeutic activity

Assays of marker substances

Markers are chemically defined constituents of a herbal drug which are of interest for control purposes

Other tested

- *microbiological quality*
- *residues of pesticides and fumigation agents*
- *radioactivity*
- *toxic metals*
- *likely contaminants and adulterants*

Analytical procedures not given in a pharmacopoeia should be validated

4.1.2 Proposals for revision of the control of herbal drug preparations

Description and validation of the manufacturing process for the herbal drug preparation

Quantitative determination of markers or of substances with known therapeutic activity is required

4.2 Proposals for revision of control tests on the finished product

Qualitative and quantitative determination of the composition of

- *markers*
- *constituents of known therapeutic activity*

If a herbal medicinal product contains several herbal drugs or preparations of several herbal drugs

Determination may be carried out jointly for several active substances.

4.3 Proposals for revision of stability tests

Herbal drug or herbal drug preparation is the active substance

- *Appropriate fingerprint chromatograms*
- *Other substances are stable*
- *Proportional content remains constant*

Herbal medicinal product contains several herbal drugs or preparations

Stability is determined by appropriate fingerprint chromatograms

5. Regulatory proposals dealing with the safety of herbal medicines

5.1 Pre-Clinical testing

Should be directed towards the study of effects that are difficult to detect clinically

Toxicity to

- *Reproduction*
- *Genotoxicity*
- *Carcinogenicity*

5.2 Expert report

6. Regulatory proposals dealing with efficacy of herbal medicines

GCP

6.1 Presentation of results

Precise composition and specification of the product investigated must be provided

6.2 Pharmacodynamics

6.3 Pharmacokinetics

If there is a constituent with known therapeutic activity and a narrow therapeutic window, pharmacokinetic data will be required

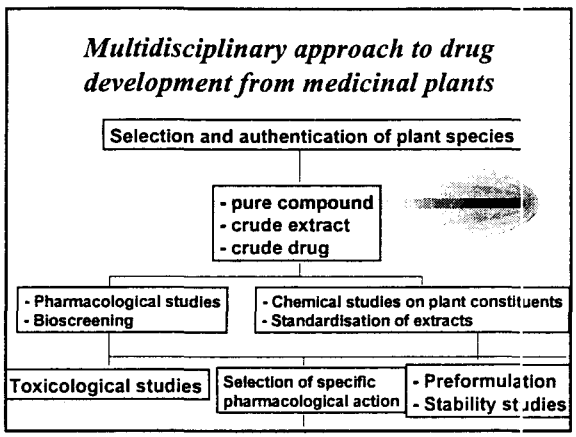
6.4 Clinical efficacy and safety

Post-marketing experience for individual products and for all related products originating from the same herbal drug

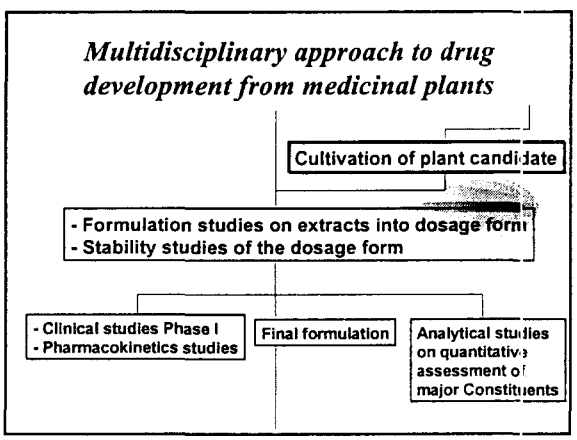
Commercialization of medicinal plants

- Phase 1: Design, Research and development
- Phase 2: Design, Production and Marketing
- Phase 3: Design, Sales and Distribution

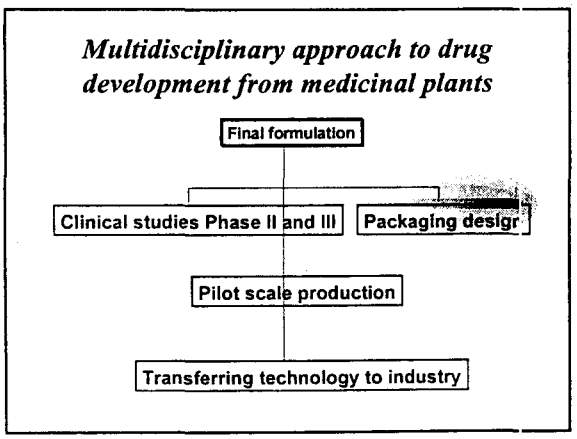
Multidisciplinary approach to drug development from medicinal plants



Multidisciplinary approach to drug development from medicinal plants



Multidisciplinary approach to drug development from medicinal plants



Special Considerations for Manufacturing

- *Raw material pre-treatment*
  - *concentration and availability of actives*
- *Generation of hazardous wastes*
  - *wastes with low solvent content*
- *Low number of unit operations*
  - *< 5% yield loss/operation*
  - *minimum 60% yield, optimum 85%*

Special Considerations for Manufacturing

- *Nature of product*
  - *thermal and chemical stability*
  - *isomeric forms*
- *Worker Safety*
  - *cytotoxins*
  - *allergens*
  - *volatile solvents*

Major Cost Factors in cGMP Manufacture of Plant Medicinals

- *Raw materials (actives content<0.1%)*
- *Capital cost of chromatography equipment*

Major Cost Factors in cGMP Manufacture of Plant Medicinals

- *Capital and operational costs for Environmental, Health and Safety*
  - *Fire control*
  - *worker exposure*
  - *HVAC systems*
  - *Air emission and wastewater discharge*
  - *Worker training*

Major Cost Factors in cGMP Manufacture of Plant Medicinals

- *Hazardous waste disposal*
- *cGMP documentation*

Quality assessment of phytopharmaceuticals

- **Assessment of crude plant material**
  - **General description of the plant**
  - **Part used**
  - **Production of crude drugs**
    - **Cultivation**
    - **Harvesting**
    - **Post-harvest handling**
    - **Packaging and storage**

Quality assessment of phytopharmaceuticals

- **Assessment of crude plant material**
  - Quality specification
    - Quality specification
    - Chemical/chromatographic identification
    - Foreign organic matter limit
    - Ash content
    - Acid-insoluble ash content
    - Water-soluble extractive
    - Alcohol-soluble extractive

Quality assessment of phytopharmaceuticals

- **Assessment of crude plant material**
  - Quality specification
    - Moisture content
    - Main/active constituent content
    - Microbial limit
    - Pesticide residue limit
    - Heavy metal limit
    - Likely contaminants
    - Adulterants

Quality assessment of phytopharmaceuticals

- **Assessment of finished product at intermediate stage of the manufacturing process**

  Same as described for finished products

Quality assessment of phytopharmaceuticals

- Assessment of finished product
  - For tablets
    - Weight variation
    - Disintegration time (not more than 30 minutes)
    - Identification of preservatives and active ingredients

Quality assessment of phytopharmaceuticals

- Assessment of finished product
  - For tablets
    - Determination of extractives in various solvents
    - Microbial limit
    - Heavy metals

Quality assessment of phytopharmaceuticals

- Assessment of finished product
  - For solutions
    - pH
    - Identification of preservatives and active ingredients
    - Alcohol content (not more than 15 percent)
    - Microbial limit
    - Sodium saccharin content (not more than allowable limit)

Quality assessment of phytopharmaceuticals

Assessment of finished product

For infusion

- **Weight variation**
- **Identification of preservatives and active ingredients**
- **Determination of extractives in various solvents**
- **Microbial limit**
- **Heavy metals**
- **Borax**

Quality assessment of phytopharmaceuticals

Assessment of finished product

For sugar coated tablets

Similar to tablets except for disintegration time which is not more than 1 hour

Chemical Standardization Methods - Challenges

- ***Complex and variable mixtures***
- ***Choice of compounds to quantitate***
- ***Difficult sample preparation***
- ***Lack of pure reference standards***
- ***Lack of methods with adequate tolerances by analytical chemistry standards***

Chemical Standardization Methods

- *TLC/HPTLC*
- *HPLC*
- *FTIR*

Chemical Markers

- *Specifications for raw materials*
- *Quality assurance in process control*
- *Standardization of product*
- *Obtain stability profiles*
- *Single marker vs. "fingerprint"*

Parameters of Assay Validation

- *Linearity*
- *Limits of Quantitation and Detection*
- *Precision*
- *Robustness*
- *Recovery*

Limits for microbial contamination

- *6 types of non-sterile pharmaceutical preparations*
  - *Preparations for burns and severe ulcerations*
  - *Topical preparations for broken skins, abscess, lesions, and mucous membranes*
  - *Topical preparations for intact skin*

Limits for microbial contamination

- *6 types of non-sterile pharmaceutical preparations*
  - *Preparations for oral, rectal and transdermal use*
  - *Preparations of crude drugs and mixtures of crude drugs for internal use, which will undergo a process for reduction of count before use*
  - *Other internally used preparations which contain whole or ground crude drugs*

Stability test

- *The stability of the medicinal product should be determined by appropriate fingerprint chromatograms*
- *It must be shown that interactions between the active ingredients and the excipients in the finished product are unlikely to occur*

New FDA Policy?

- " A long history of safe use might provide sufficient safety information for products that are intended for short-term use. For some thing that's given only briefly.....for short term, you may consider the history of exposure, if it can be documented recently, as relevant safety. However, (it is still) questionable whether a history of safe use would be adequate to support the safety of an ingredient intended for regular, long term use."

New FDA Policy?

- "We don't have a rule that people who carry out trials have to be physicians. I don't see why someone with (an herbal) background couldn't carry out the trial. Someone who's experienced in (herbal) use, and believes that certain things are true is probably the best person to help design the trial. You can test Eastern philosophy in a Western controlled trial."

Four FDA Policy Issues (1)

☎ *Question:*

- Will HPLC "fingerprints" and markers support composition, uniformity and reproducibility of heterogeneous, multi-component products?

✍ *Answer:*

- Yes, technology exists to assure batch to batch consistency with quality control and stability control via HPLC and other analytical techniques.

Four FDA Policy Issues (2)

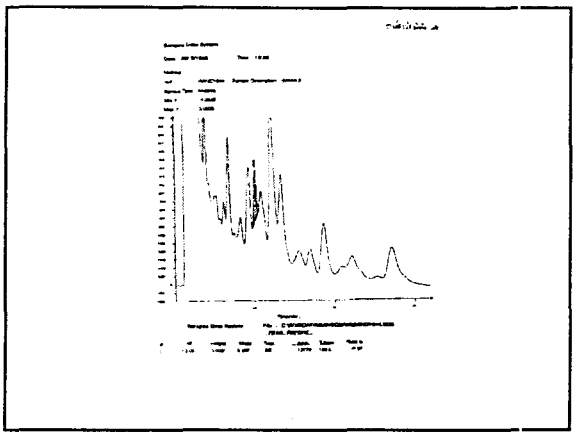
- *Question:*
  - Would FDA consider the efficacy of multi-component botanicals without requiring studies on the contribution of each ingredient to efficacy?
- *Answer :*
  - Yes, based on the approval of IND's for botanical formulas of heterogeneous multi-component ingredients FDA now appears willing to evaluate these as single therapeutic entities

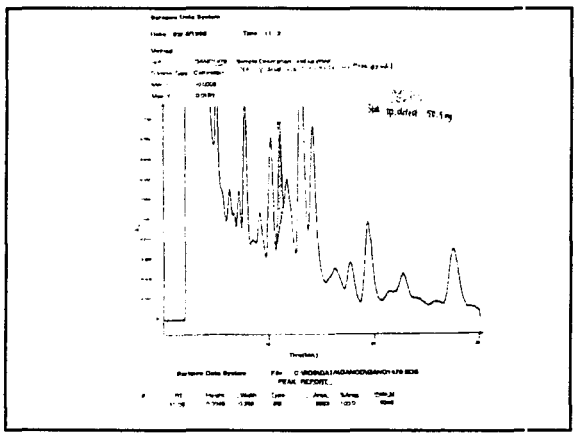
Four FDA Policy Issues (3)

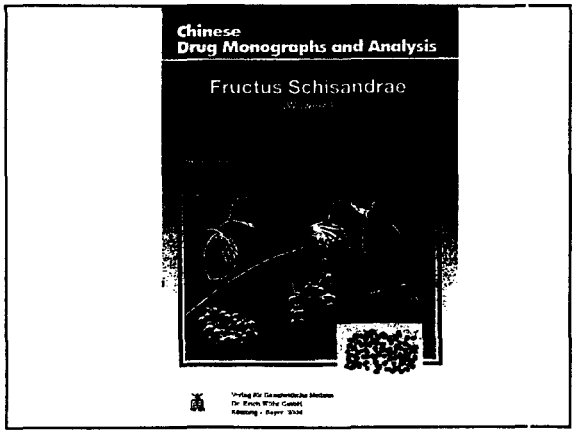
- *Question:*
  - Can FDA be convinced to consider "unevenful, long-term usage of botanicals by large numbers of humans" as an adequate predictor of safety in lieu of animal toxicological studies?
- *Answer:*
  - Are two year studies in 100 animals as predictive of human safety as hundreds of year experience in 1000's of human?

Four FDA Policy Issues (4)

- *Question:*
  - Would FDA consider long-term use combined with pilot clinical trials as sufficient to support the effectiveness of a botanical product?
- *Answer:*
  - A "general consensus" exits in support of the idea that smaller "pilot-type" clinical trials may be acceptable when accompanied by a large body of historical use data.






Chinese
Drug Monographs and Analysis
Fructus Schisandrae

IMPRESSUM

of the Book Publication

Schisandra Sphenanthera and Kadsura

Fructus Schisandrae – *Wuweizi*

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Authors and Editors

Prof. Dr. Dr. h. c. H. Wagner, Institut für Pharmazeutische Biologie, Ludwig-Maximilians-Universität München, Karlstraße 29, D-80333 München

Prof. Dr. K. Bauer, Institut für Pharmazeutische Biologie, Ruhr-Universität Bochum, Universitätsstraße 1, D-44780 Bochum

Co-Editors

Prof. Dr. Zhu Ruiyan and Prof. Dr. Chou Jianming, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences, 3 Xin Wang Road, Hai Dian District, Beijing 100094, PR China

Co-Author

Sonia Wiesinger, Institut für Pharmazeutische Biologie, Karlstraße 29, D-80333 München

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Fructus Schisandrae – *Wuweizi*

Pharmacopoeias	Pharmacopoeia of the People's Republic of China, English Edition, 1992
First cited in	Shen Nong Ben Cao Jing (?)
Official drugs	*Schisandra chinensis* (Turcz.) Baill.
	Schisandra sphenanthera Rehder & Wilson
	– Magnoliaceae –
Nomenclature of the dried fruit	Chinese pharmacognosy distinguishes the fruits of *Schisandra chinensis* (Northeast China) and *Schisandra sphenanthera* (Central China) which differ in their pharmacology and in their uses. *Schisandra sphenanthera* from North China, while synonymous to *wuweizhi*, is to *schisandra pubescens*.
Schisandra chinensis	The berries are 5-6 mm in diameter, the outer surface is red or violet-red, wrinkled and with soft pulp. The 1-2 seeds are brownish-yellow and reniform.
Schisandra sphenanthera	The fruits are smaller and have brownish-red or dark-brown colour
Substitute drugs	Kadsura berries, *Kadsura* and *Illicium* species.
Preparation of the drug	Collected and dried (often sulphurized), crushed with vinegar (sometimes)
Medicinal use	In traditional medicine in China and Japan as antihepatotoxic, antitussive, antipyretic, antidiarrhoic, sedative and tonic drug and also in cases of alcohol.

Effects and indications according to Traditional Chinese Medicine (TCM)	
Taste	sour
Temperature	warm
Channels entering	lung, spleen, heart, liver
Effects	astringent, tonic, tonifies the kidney and the spleen
Symptoms, indications	chronic cough, shortness of breath, consumption, night sweating, spermatorrhoea, loss of appetite, chronic diarrhoea, enuresis caused by heat, polyuresis, incontinence, nocturia caused by internal heat

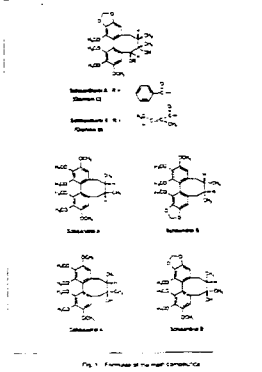


Fig. 1: Formulae of the main components

Main constituents (see Fig. 1):

- dibenzocyclooctadiene lignans (schisandrin A/B, schisandrin A/B/C, schisandrol A-E, gomisin, angeloylgomisin, tigloylgomisin, wuweizisu, schisandrin)
- monoterpenes (borneol, 1,8 cineol, citral, p-cymol, α,β-pinene)
- sesquiterpenes (ar-curcumene, (+)-α-ylangene, ar-turmerol, α- and β-chamigrene, β-bisabolene) (6,8)

In addition, vitamins C and E, fumaric acid, phytosterol (1, 7)

Pharmacology:

in vitro effects:

- antioxidative activity (8)
- antibacterial (9)

in vivo effects:

- hepatoprotective effects, SGPT-lowering effects (1)
- antiinflammatory effects on the mammalian and intestinal tract (2)
- stimulating lung function (1)
- antiasthmatic effect (11)
- cardiovascular effects on the systole (1)
- stimulation of the uterus (1)
- neuroleptic (7)
- antibacterial (7)

TLC fingerprint analysis:

1) Extraction:
6 g air dried/dry ground drug, in pounce-extracted for 2 hrs with 150 ml methanol p.a.
The extract is filtered and the filtrate concentrated in vacuum.
15,0 ml. The residuum is filled up to 15,0 ml with methanol p.a.

2) Standards:
Schisandrin A and B, schisandrin A, schisandrol A and B, stigmasterol
(1 mg dissolved in 1 ml methanol p.a.)

3) Separation parameters:
Applied amount: 20 µl extract
Plates: Silicagel 60 F 254, Merck
Solvent system: toluene + ethyl acetate + glacial acetic acid (75 + 22 + 3)
(lignans, terpenes)

Direct evaluation:
UV 254 nm and UV 366 nm

Spray reagents:
Anisaldehyde-sulphuric acid reagent (0,1 ml anisaldehyde + 10 ml glacial acetic acid 96 %
methanol + 5 ml conc. sulphuric acid are mixed in this order).

The plate is sprayed (approx. 15 ml) and heated for 5 – 10 min. at 105°C. The evaluation is carried out in vis.

Drug samples:

1	Commercial drug from China	*Schisandra sphenanthera*
2	Commercial drug from China (Sichuan Province)	*Schisandra sphenanthera*
3	Commercial drug from China (Hebei Province)	*Schisandra chinensis*
4	Commercial drug from Japan	*Schisandra chinensis*

Test substances:

T1 Schisandrin B
T2 Schisandrin A
T3 Stigmasterol
T4 Schisandrin A
T5 Schisandrin A/Schisandrol B

Fig. 2:
Thin layer chromatogram of Schisandrae fructus

4) Description of the chromatograms:

UV 254 nm:

In UV 254 nm, the lignans, distributed over the entire Rf range, can be detected as spots which partially quench fluorescence.

Drug samples 1 and 3 are lacking the lignans schisandrol A and B.

Anisaldehyde-sulphuric acid reagent, vis:

The minor lignans, schisandrin B, schisandrin A, schisandrin A, schisandrol B, and schisandrol A appear in the Rf range 0,3 – 0,6.

Schisandrin B (Rf 0,6) exhibits a light orange colour, schisandrin A (Rf 0,6) a green-grey and schisandrin A (Rf 0,45) a brown colour, while schisandrol B (Rf 0,4) and schisandrol A (Rf 0,3) show a pink-violet colour.

Samples 2 and 4 (*Schisandra sphenanthera*) are characterized by the lignans schisandrin A/B and schisandrol A/B. In samples 1 and 3 (*Schisandra sphenanthera*), schisandrin A/B and schisandrol A/B are lacking. Schisandrin A can be found only in samples 2 and 4. All samples contain minor lipid components (triglycerides) (Rf 0,9 – 1,0 and Rf 0,8 – 0,7). They are especially distributing in samples 1 and 2. The chromatogram of sample 3 shows the highest amount of starch.

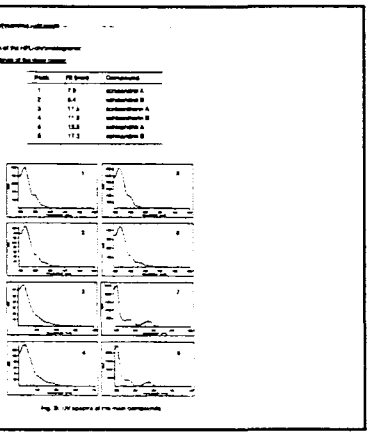
HPLC fingerprint analysis:

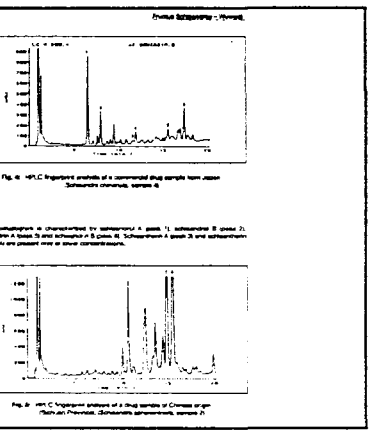
1) Sample preparation: Aliquot of the extract used for TLC (max. 50µl/spot® Micropipette and Type HR 0-50 mm.

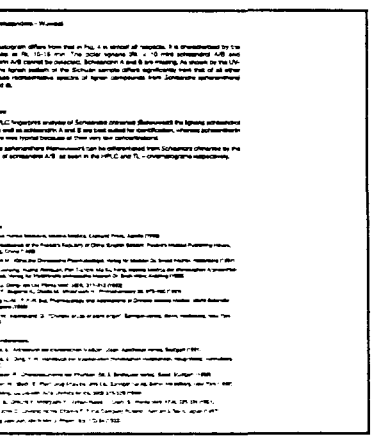
2) Injection volume: 7 µl methanolic vapi extract (conc. = 5 g drug/15 ml)

3) HPLC data:

Apparatus:	Liquid chromatograph HP 1090 Photodiode array detector HP 1040 A, Hewlett Packard
Column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent system:	A: Wasser (bidest.) + 1 % (V/V) H₃PO₄ B: acetonitrile p.a. + 1 % (V/V) 0,1 %ige H₃PO₄
Gradient:	linear +0-80 % B + 30 min
Flow:	1,0 ml/min
Detection:	210 nm







STANDARDISATION & QUALITY CONTROL OF MEDICINAL & AROMATIC PLANTS & THEIR PRODUCTS

Dr. Krisana Kraisintu

HPLC METHOD VALIDATION

Dr. Krisana Kraisintu
Research and Development Institute
Government Pharmaceutical Organization

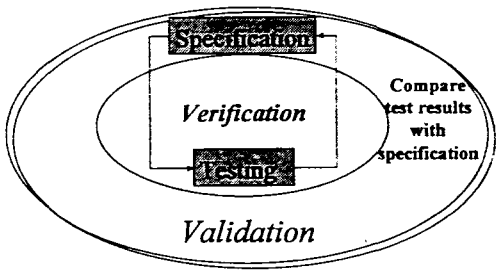
Definitions of Methods

- Official/Standard methods
  ISO, AOAC, USP, Company standards
- Developed in house for routine use
  Based on literature, in house experience, or from colleges

Definitions of Methods

- Developed in house generic methods
  For single samples, parameters are not fixed validation is different, system suitability testing is the same

Validation, Verification and Testing



Validation

- establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification (*Source: FDA guidelines on General Principles of Validation, March 1986*)

Capacity Factor

$$k' = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}} = \frac{t_R - t_M}{t_M}$$

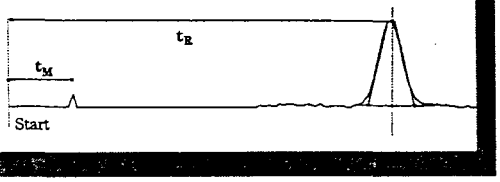
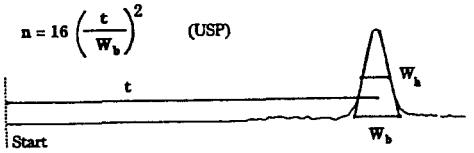


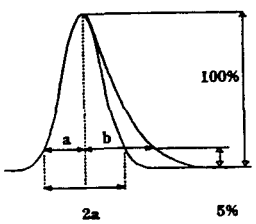
Plate Number

$$n = 5.545\left(\frac{t}{W_h}\right)^2$$ (British Pharmacopoeia)

$$n = 16\left(\frac{t}{W_b}\right)^2$$ (USP)



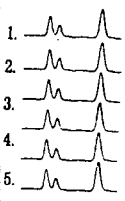
Tailing Factor (Asymmetry Factor)



USP: Ratio of the distance from the leading edge of the peak at 5% peak height divided by twice the distance from the peak maximum to the leading edge.

$$A = \frac{a + b}{2a}$$

Precision (Repeatability)



$$S_R(\%) = \frac{100}{\bar{X}}\left[\frac{\sum_{i=1}^{N}(X_i - \bar{X})^N}{(N-1)}\right]$$

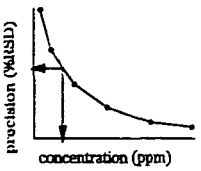
S_R = Relative standard deviation in percentage

$\bar{X}$ = mean of a set of N measurements

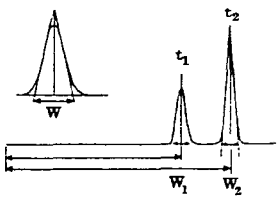
X_i = individual measurement

Method Detection/Quantitation Limits

- Define expected precision for detection/quantitation limit
- Prepare spiked matrix samples
- Dilute and inject 6 times
- Calculate %RSD for each concentration
- Plot precision vs. concentration
- Concentration at expected precision = limits



System Resolution



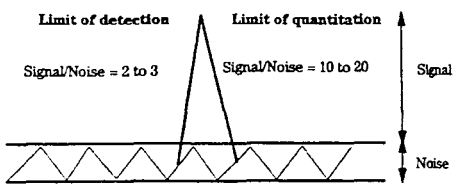
$$R = \frac{2\,(t_2 - t_1)}{W_2 + W_1}$$

t = elution time

W = peak width at base

Should be measured as part of system suitability testing (USP)

Limit of Peak Detection and Quantitation in Chromatography



Method Validation

When
? During and at end of method development
? After modifications

Requirement
? Analysis methods shall be validated and results need to be documented
? Results shall be independent from instrument, (laboratory), and operator

Method Validation

Precision

Accuracy

Linearity

Selectivity

Limit of detection

(Stability)

Limit of quantitation

Ruggedness

Environmental
-Sensitivity-
-Selectivity-

Pre-dosage
-Precision-

Post-dosage
(biological samples)
- Selectivity-

Actual validation effort depends on the analysis problem

What do you want to detect-in which matrix-at which detection limits

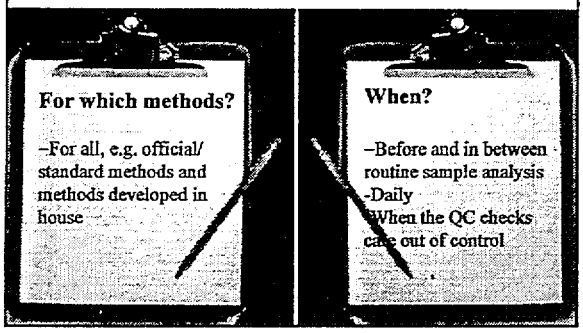
Steps in Method Validation

- ωDevelop validation plan
- ωDefine purpose of method (performance criteria)
- ωVerify performance of instrument
- ωQualify/train operator

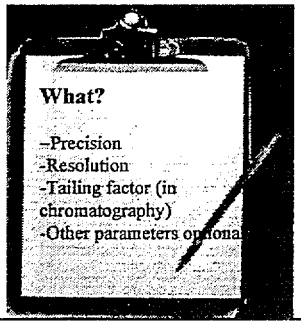
Steps in Method Validation

- ωQualify/validate materials
- ωPerform validation experiments - internal/external
- ωDevelop SOPs for executing the method
- ωDefine system suitability tests and QC checks
- ωDocument everything

System Suitability Testing



System Suitability Testing



Linearity

Ability of the method to obtain results which are directly,or by means of mathematical calculations, proportional to the concentration of the analyte

Calculation of the regression line of the response vs. the concentration

Selectivity vs. Specificity

Specific
Produces response for single analyte

Selective
Produces response for a number of analytes which can be distinguished

USP definition of selectivity: Ability to measure accurately an analyte in the presence of interferences

Methods to Validate Selectivity

- Calibration curve through 0
- Accuracy: if accurate ? also selective
- Chromatography
  - resolution
  - peak shape
  - different columns
  - different conditions (temp., mobile phase)
  - different detectors

Ruggedness

Degree of variance in test results under different test conditions

- Within one laboratory
- Within different laboratories

Factors affecting ruggedness

- Different room temperature and humidity
- Analysts with different experience
- Instruments with different characteristics
- Reagents from different suppliers
- Columns from different batches

Stability

Replicate analysis of the same sample solution

1. Determine system precision (replicate short term analyses)
2. Determine system stability (replicate long term analyses, for example every 24 or 48 hours, should include freeze/thaw cycles)

System stability is appropriate if results do not exceed 20% of system precision

Method Re-validation

When

1. Method parameters have been changed
2. The scope of the method has been changed (for example from one specific type of GC instrument to general Gaschromatograph)

Method Re-validation

What

Preferably everything. Exceptions should be scientifically justified

Thank you

ISO/IEC GUIDE 25

Dr. Krisana Kraisintu

Research and Development Institute

Government Pharmaceutical Organization

What is Metrology?

- Metrology is the science of measurement

What is Calibration?

- Calibration is the comparison of measuring and test equipment or measurement standard of unknown accuracy to a measurement standard of known accuracy in order to detect, correlate, report, or eliminate by adjustment any variation in the accuracy of the instrument being compared.

What is Traceability?

Traceability is the ability to relate individual measurement results through an unbroken chain of comparisons to a standard.

Standard

An instrument, device, or material of known characteristics and higher precision used to establish and maintain the accuracy of a measurement system or device.

Accuracy

The degree of correctness, closeness of its result to the true value, usually expressed in terms of 'ERROR'.

Uncertainty

A part of the expression of result of the measurement which states the range of values within which the true value is estimated to lie.

?SYSTEMATIC UNCERTAINTY

?RANDOM UNCERTAINTY

Precision

- A measure of the consistency or reproducibility of measurements among themselves.
- A high precision indicates ability to repeat measurements with narrow limits.

Error

- Error is the discrepancy between the result of measurement and the true value of the quantity being measured.

Error

- **Gross errors**
- **Systematic errors**
- **Random errors**

Errors which are strickly under the control of the individual, totally separate from the instrumentation.

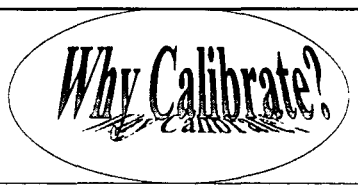
- Misreading of instrument
- Marking incorrect adjustment
- Applying instrument improperly
- Computational errors

Systematic Errors

- Errors relate to the instrumentation of external influences to the instrument. They cause the measured value to be offset by a fixed amount.
- The smaller SYSTEMATIC ERROR
- The higher ACCURACY

Random Errors

- **Errors which are indicated as a scatter about an average when a multiple number of measurements are taken.**
- **The smaller RANDOM ERROR**
- **The higher PRECISION**



- Calibration is part of a good quality control system
- Provide a product or service which MEETS or EXCEEDS your customer's expectations

- All instruments and tools change with
  - Time
  - Temperature
  - Humidity
  - Environmental ExposureNormal Use (Wear and Tear)
  - Abuse
- No two instruments change in the same manager

What properties are calibrated?

All important measured parameters:

- ✓Electrical
- ✓Dimensional
- ✓Temperature
- ✓Pressure
- ✓Time
- ✓Acoustical

Definition of Traceability

- Unbroken chain of calibration measurements from an Instrument to National Standards
- With documented proof of the chain of calibrations

Why is traceability important?

? Quality Assurance :

- Uniformity of manufactured goods
- Uniformity of industrial processes
- Tolerances are met
- Supports ISO 9000

Why is traceability important?

? Compliance:

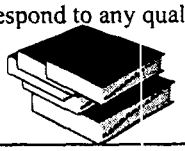
- **OSHA 1910, FDA, GMP, & Others**
- **Contracts**

Benefits of Calibration

- ▲Calibration insures all independent processes are working to the same set of standards
- ▲Calibration insures these processes stay working to the same set of standards
- ▲Calibration supports product quality and save money
- ▲A good calibration program is a fundamental part of a good quality control program

Why document your calibrations?

- Documented Procedures establish a baseline for proper and consistent calibration
- Documented Results provide the historical information to manage the quality control process and to properly respond to any quality changes



Appendix B

Calibration intervals and performance checks

General comments on laboratory equipment by type

RDI QUALITY CONTROL LABORATORY — Month January

CALIBRATION REPORT OF pH METER — Page 1 of 4

CALIBRATION REPORT OF pH METER

Dept : RDI QUALITY CONTROL LABORATORY

Month : January

Make : WTW — Model : pH 3000

Serial No. : 4132011

Model of electrode : E56

Type of buffer : Technical Buffer — Make : Orion

Mode : AutoCal Variabel

Buffer 1 : 4.01

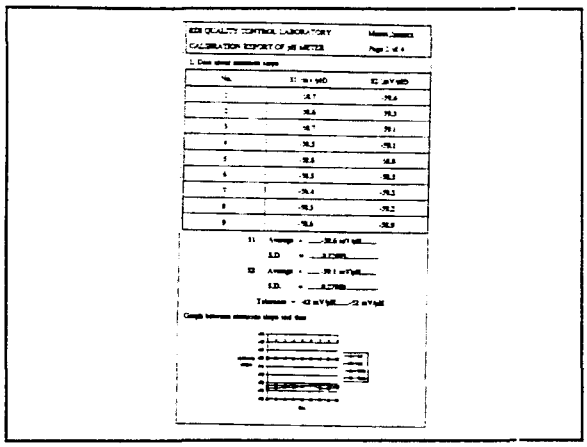
Buffer 2 : 7.00

Buffer 3 : 10.01

RESULT — (PASS) — FAIL

Calibrated By .... Approved By ....

Date ....



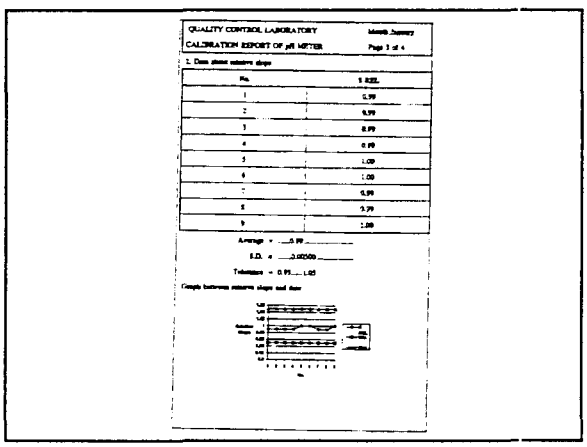
RDI QUALITY CONTROL LABORATORY — Month January
CALIBRATION REPORT OF pH METER — Page 1 of 4

1. Does slope maintain span

No.	S1 -ve mV	S2 +ve mV
1	-58.7	59.6
2	-58.6	59.3
3	-58.7	59.1
4	-58.3	-59.1
5	-58.8	58.8
6	-58.3	58.3
7	-58.4	-59.3
8	-58.3	-59.2
9	-58.6	-58.9

S1 Average = —58.6 mV/pH—
S.D. = —0.17889—
S2 Average = —59.1 mV/pH—
S.D. = —0.27889—
Tolerance = -52 mV/pH——52 mV/pH

Graph between minimum slope and date



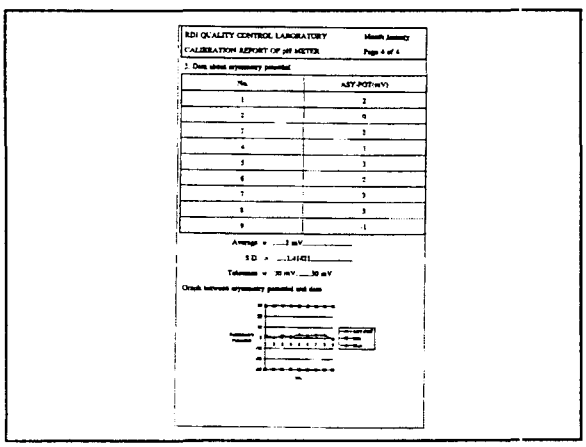
QUALITY CONTROL LABORATORY — Month January
CALIBRATION REPORT OF pH METER — Page 3 of 4

2. Does slope minimum slope

No.	S REL
1	0.99
2	0.99
3	0.99
4	0.99
5	1.00
6	1.00
7	0.99
8	0.99
9	1.00

Average = —0.99—
S.D. = —0.00500—
Tolerance = 0.95——1.05

Graph between minimum slope and date



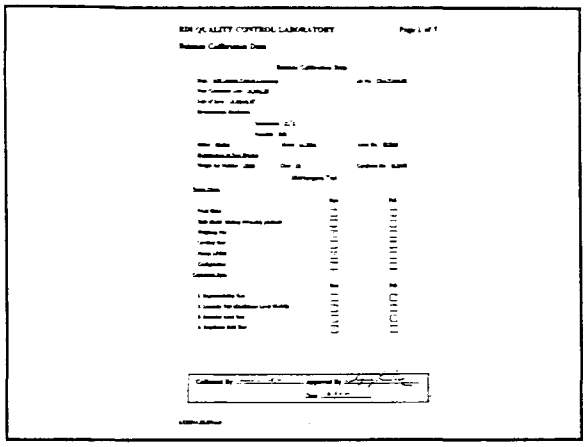
RDI QUALITY CONTROL LABORATORY — Month January
CALIBRATION REPORT OF pH METER — Page 4 of 4

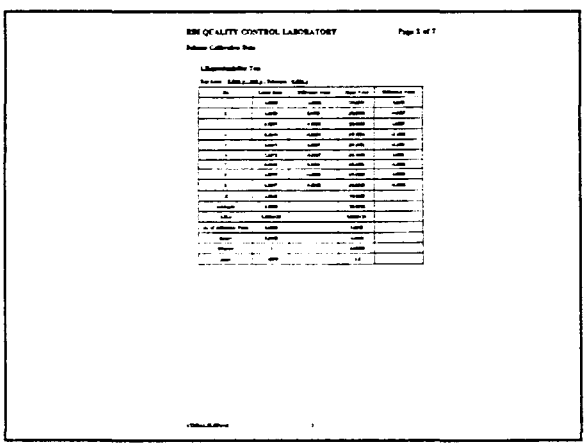
3. Does slope asymmetry potential

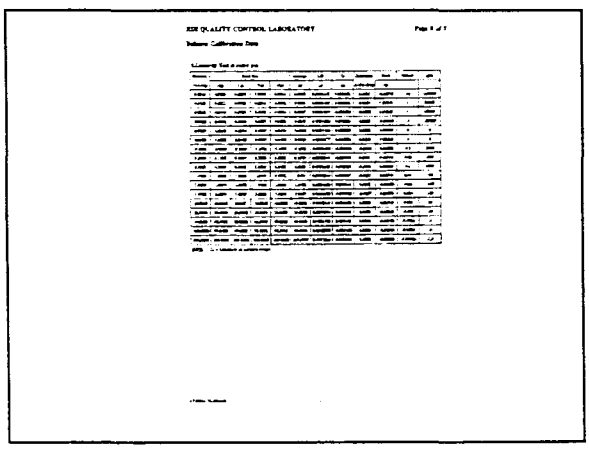
No.	ASY-POT (mV)
1	2
2	0
3	3
4	1
5	3
6	2
7	3
8	3
9	1

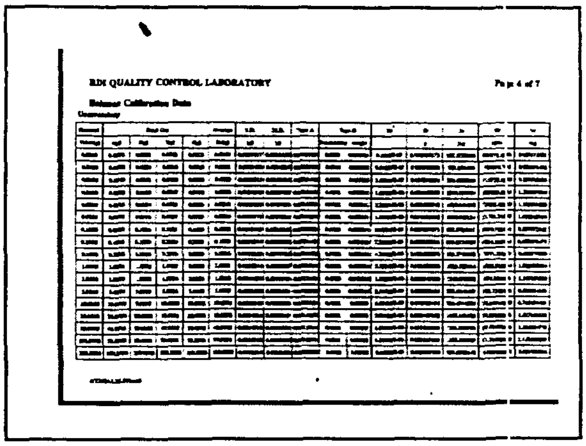
Average = —2 mV—
S.D. = —1.41421—
Tolerance = 30 mV ——30 mV

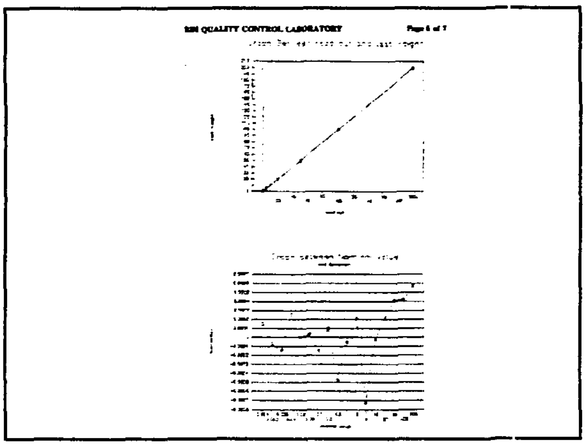
Graph between asymmetry potential and date

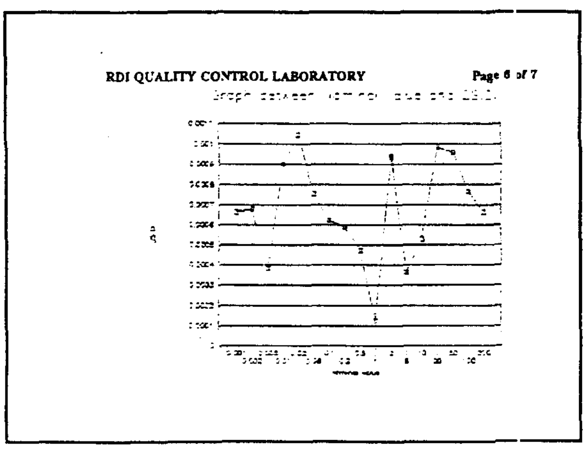


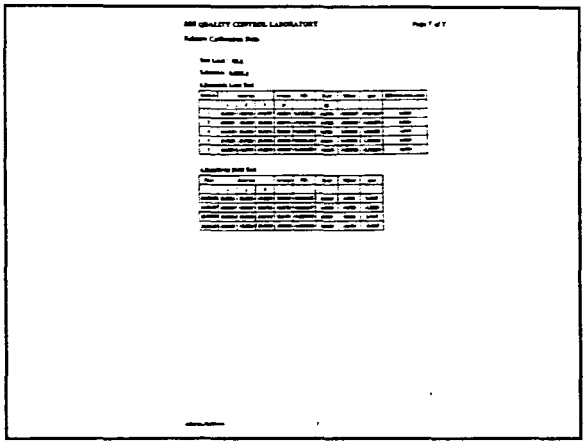


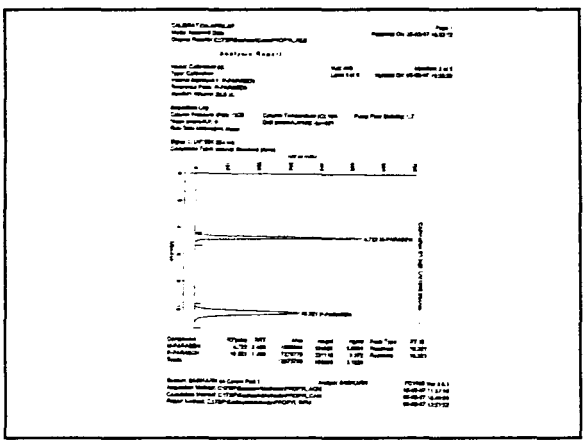


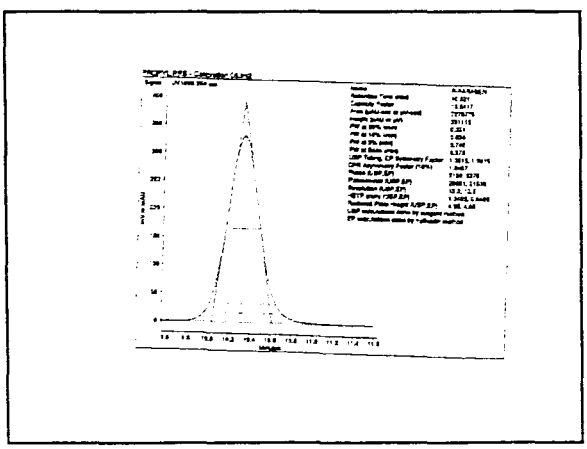

RDI QUALITY CONTROL LABORATORY
Page 4 of 7
Balance Calibration Data

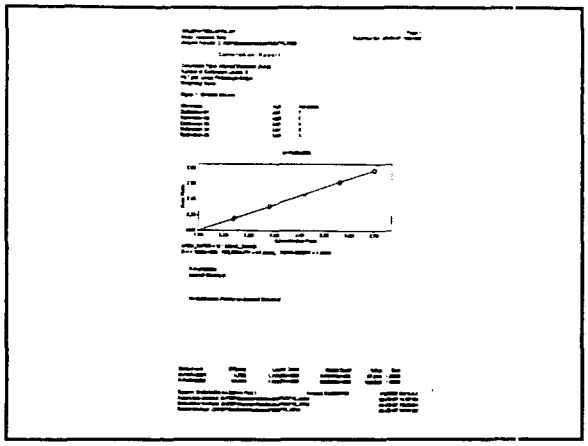

RDI QUALITY CONTROL LABORATORY

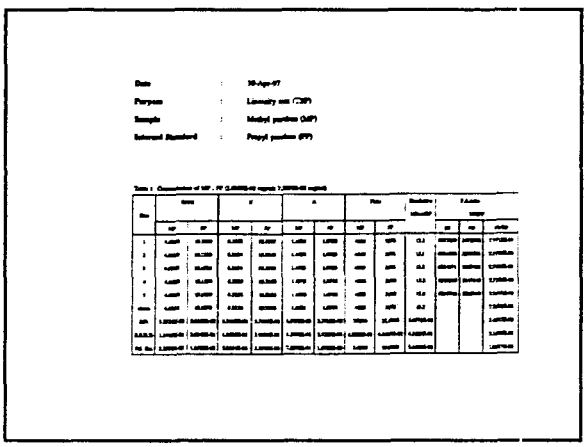

RDI QUALITY CONTROL LABORATORY
Page 6 of 7



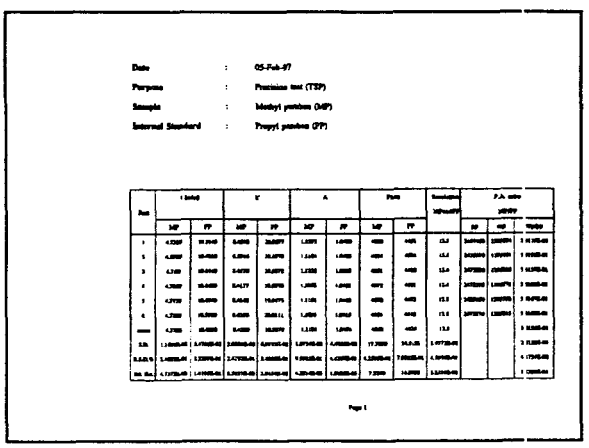




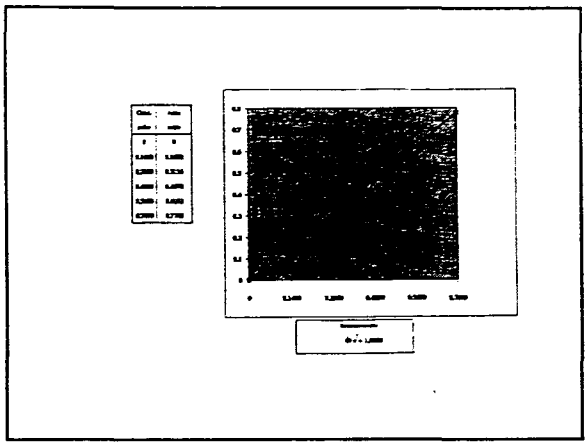




Date	:	30-Apr-97
Purpose	:	Linearity test (TSP)
Sample	:	Methyl parathion (MP)
Internal Standard	:	Propyl parathion (PP)



Date	:	05-Feb-97
Purpose	:	Precision test (TSP)
Sample	:	Methyl parathion (MP)
Internal Standard	:	Propyl parathion (PP)



Appendix A

Quality audit : areas of particular importance to a chemistry laboratory

A1 Staff

A2 Environment

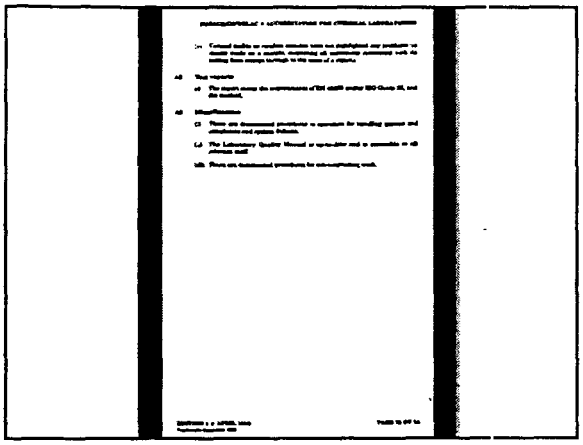
A3 Methods and procedures

A4 Standards, calibrations and controlled substances

A5 Quality Control

A6 Sample management

A7 Records



Thank you

Quality assurance of pharmaceuticals
A compendium of guidelines and related materials, Volume 1
World Health Organization 1997

Guidelines for the assessment of herbal medicines

Introduction

For the purpose of these guidelines, herbal medicines are defined as follows:

> Finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substances of this nature. Herbal medicines may contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be herbal medicines.
>
> Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin.

The objective of these guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines and thereby to assist national regulatory authorities, scientific organizations and manufacturers to undertake an assessment of the documentation/submissions/dossiers in respect of such products. As a general rule in this assessment, traditional experience means that long-term use as well as the medical, historical and ethnological background of those products shall be taken into account. The definition of long-term use may vary according to the country but should be at least several decades. Therefore, the assessment should take into account a description in the medical/pharmaceutical literature or similar sources, or a documentation of knowledge on the application of a herbal medicine without a clearly defined time limitation. Marketing authorizations for similar products should be taken into account.

Assessment of quality

Pharmaceutical assessment

This should cover all important aspects of the quality assessment of herbal medicines. It should be sufficient to make reference to a pharmacopoeial monograph if one exists. If no such monograph is available, a monograph must be supplied and should be set out as in an official pharmacopoeia.

All procedures should be in accordance with good manufacturing practices.

Crude plant material

The botanical definition, including genus, species and authority, should be given to ensure correct identification of a plant. A definition and description of the part of the plant from which the medicine is made (e.g. leaf flower, root) should be provided, together with an indication of whether fresh, dried or traditionally processed material is used. The active and characteristic constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

Plant preparations

Plant preparations include comminuted or powdered plant materials, extracts, tinctures, fatty or essential oils, expressed juices and preparations whose production involves fractionation, purification or concentration. The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a certain level of active or characteristic constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g. "chromatographic fingerprint") to ensure consistent quality of the preparation.

Finished product

The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined. A method of identification and, where possible, quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g. "chromatographic fingerprint") to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

Stability

The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established.

Assessment of safety

This should cover all relevant aspects of the safety assessment of a medicinal product. A guiding principle should be that, if the product has been traditionally used without demonstrated harm, no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk–benefit assessment.

A review of the relevant literature should be provided with original articles or references to the original articles. If official monograph/review results exist, reference can be made to them. However, although long-term use without any evidence of risk may indicate that a medicine is harmless, it is not always certain how far one can rely solely on long-term usage to provide assurance of innocuity in the light of concern expressed in recent years over the long-term hazards of some herbal medicines.

Reported side-effects should be documented according to normal pharmacovigilance practices.

Toxicological studies

Toxicological studies, if available, should be part of the assessment. Literature should be indicated as above.

Documentation of safety based on experience

As a basic rule, documentation of a long period of use should be taken into consideration when assessing safety. This means that, when there are no detailed toxicological studies, documented experience of long-term use without evidence of safety problems should form the basis of the risk assessment. However, even in cases of drugs used over a long period, chronic toxicological risks may have occurred but may not have been recognized. The period of use, the health disorders treated, the number of users and the countries with experience should be specified. If a toxicological risk is known, toxicity data must be submitted. The assessment of risk, whether independent of dose or related to dose, should be documented. In the latter case, the dosage specification must be an important part of the risk assessment. An explanation of the risks should be given, if possible. Potential for misuse, abuse or dependence must be documented. If long-term traditional use cannot be documented or there are doubts on safety, toxicity data should be submitted.

Assessment of efficacy

This should cover all important aspects of efficacy assessment. A review of the relevant literature should be carried out and copies provided of the original articles or proper references made to them. Research studies, if they exist, should be taken into account.

Activity

The pharmacological and clinical effects of the active ingredients and, if known, their constituents with therapeutic activity should be specified or described.

Evidence required to support indications

The indication(s) for the use of the medicine should be specified. In the case of traditional medicines, the requirements for proof of efficacy should depend on the kind of indication. For treatment of minor disorders and for non-specific indications, some relaxation in requirements for proof of efficacy may be justified, taking into account the extent of traditional use. The same considerations may apply to prophylactic use. Individual experiences recorded in reports from physicians, traditional health practitioners or treated patients should be taken into account.

Where traditional use has not been established, appropriate clinical evidence should be required.

Combination products

As many herbal remedies consist of a combination of several active ingredients, and as experience of the use of traditional remedies is often based on combination products, assessment should differentiate between old and new combination products. Identical requirements for the assessment of old and new combinations would result in inappropriate assessment of certain traditional medicines.

In the case of traditionally used combination products, the documentation of traditional use (such as classical texts of Ayurveda, traditional Chinese medicine, Unani, Siddha) and experience may serve as evidence.

An explanation of a new combination of well known substances, including effective dose ranges and compatibility, should be required in addition to the documentation of traditional knowledge of each single ingredient. Each active ingredient must contribute to the efficacy of the medicine.

Clinical studies may be required to justify the efficacy of a new ingredient and its positive effect on the total combination.

Intended use

Product information for the consumer

Product labels and package inserts should be understandable to the consumer or patient. The package information should include all necessary information on the proper use of the product.

The following elements of information will usually suffice:

- name of the product
- quantitative list of active ingredient(s)
- dosage form
- indications
  - dosage (if appropriate, specified for children and the elderly)
  - mode of administration
  - duration of use
  - major adverse effects, if any
  - overdosage information
  - contraindications, warnings, precautions and major drug interactions
  - use during pregnancy and lactation
- expiry date
- lot number
- holder of the marketing authorization.

Identification of the active ingredient(s) by the Latin botanical name, in addition to the common name in the language of preference of the national regulatory authority, is recommended.

Sometimes not all information that is ideally required may be available, so drug regulatory authorities should determine their minimal requirements.

Promotion

Advertisements and other promotional material directed to health personnel and the general public should be fully consistent with the approved package information.

Utilization of these guidelines

These guidelines for the assessment of herbal medicines are intended to facilitate the work of regulatory authorities, scientific bodies and industry in the development, assessment and registration of such products. The assessment should reflect the scientific knowledge gathered in that field. Such assessment could be the basis for future classification of herbal medicines in different parts of the world. Other types of traditional medicines in addition to herbal products may be assessed in a similar way.

The effective regulation and control of herbal medicines moving in international commerce also requires close liaison between national institutions that are able to keep under regular review all aspects of production and use of herbal medicines, as well as to conduct or sponsor evaluative studies of their efficacy, toxicity, safety, acceptability, cost and relative value compared with other drugs used in modern medicine.

Good laboratory practices in governmental drug control laboratories[1]

1. General

Correct assessment of the quality of a drug sample is dependent on:

— the submission of a representative sample to the laboratory, together with a precise indication of why the test is requested;
— a correctly planned and meticulously executed analysis; and
— a competent evaluation of the results to determine whether the sample complies with the specification.

Precise documentation and efficient routines are required to make each operation as simple and as foolproof as possible.

These guidelines provide advice on the analysis both of dosage forms and of pharmaceutical raw materials; particular consideration is given to developing countries wishing to establish governmental drug control laboratories or having recently done so.

Many of the recommendations are also relevant to drug testing in pharmaceutical production plants, but this is a matter of the repetitive testing of a limited number of pharmaceutical products, whereas governmental control laboratories theoretically have to deal with all drugs on the market and therefore have to use a wider variety of test methods.

2. Management and operational issues

2.1 Organizational structure

The full analysis of a drug sample involves a variety of different tests. In a small laboratory where relatively few analyses are undertaken a single analyst may have to take responsibility for carrying out all the chemical and physicochemical tests and evaluating the results. In large laboratories, on the other hand, the sample may be subdivided between several specialized subunits, each of which carries out the part of the analysis that calls for the particular skills and technology that it possesses. In every case, however, a "lead unit" or focal point must be made responsible for distributing and testing the sample and collating and interpreting the results.

The division of the laboratory into subunits may be based on the main techniques used (e.g. chemical unit, instrumental unit, microbiological unit, unit for biological safety testing) or on the type of product tested (e.g. antibiotics unit, crude drug unit, radiopharmaceuticals unit). Whichever plan is chosen, care must be taken to ensure an even distribution of the workload between units and the precise allocation of responsibilities, particularly in the designation of lead units for particular types of drugs. Units specializing in single assay techniques, such as sterility testing, pyrogen testing or special physical measurements, should be regarded as collaborating units that perform specific tests at the request of the lead unit.

Division of a laboratory into subunits should never be allowed to inhibit communication between the staff involved in testing the same sample. Intercommunication helps the lead unit to piece together all the information on which the quality of the sample is ultimately judged.

Large laboratories need various supporting and coordinating sections, including a central registry and a specifications repertory. The size of these units will depend on the number of samples received and the number of different drugs subjected to testing. The head of the central registry must be a person with wide experience in analysis and will be responsible for receiving all incoming samples and accompanying documents, supervising their delivery to the lead units and keeping a constant check on the progress of analyses and the despatch of completed reports. He or she may also be required to collate and evaluate the test results for each analysis. The specifications repertory section maintains an up-to-date collection of all quality specifications and related documents.

2.2 Staffing

The head of the laboratory, and the heads of the various subunits in larger establishments, should be of high professional standing and have had extensive previous experience in drug analysis and laboratory management in a quality control laboratory in the regulatory sector or in industry. Non-supervisory analysts should be graduates in pharmacy, analytical chemistry, microbiology, or other relevant subjects. Technical staff should preferably hold diplomas in their subjects from technical or vocational schools.

The head of the laboratory must be satisfied that all key members of the laboratory staff have the requisite competence and are given grades matching their responsibilities. To encourage them in carrying out their tasks, all staff should be made aware of the important contribution of drug control to public health. In many countries, national regulations prohibit staff from holding independent posts or consultant assignments.

To reduce the possibility of human error, supervisors should periodically arrange for standard samples to be analysed and, where called for, review the adequacy of existing staffing, management, and training procedures. Error is most likely to occur during non-instrumental operations, and particularly in preparatory work, from carelessness, fatigue, boredom, inadequate training or, sometimes, as a result of staff being given work beyond their level of competence. "Self-checking" procedures should be devised for instrument operators. Regular in-service training programmes should be arranged to update and extend the skills of both professionals and technicians. This not only keeps staff abreast of advances in analytical methods and instrumentation, but also provides opportunities for career development and promotion.

In large laboratories the staffing of the various units should be based not only on their workloads but also on the technical demands of the work involved. In most instances the ratio of technicians to analysts should be 1 : 3 in a chemical or physicochemical unit, and 2 : 5 in a biological or microbiological laboratory. The greater the proportion of routine analyses undertaken on products that, *a priori*, are not expected to be substandard, the greater the proportion of technicians that can be effectively employed. Non-routine work, and particularly the review of test methods for newly registered drugs, requires a higher proportion of fully qualified analysts.

2.3 Incoming samples

As an initial step in quality evaluation, each incoming sample and the accompanying documents should be numbered and logged in a central register, which may be a record book, a card file, or data processing equipment. The entry should indicate the date when the sample was received and the lead unit to which it was forwarded. To facilitate the routing and tracing of samples, a list of the lead units assigned to each drug on the market should be kept in the central registry. Any unlisted products can then be assigned on a case-to-case basis by the head of the laboratory.

All persons, and particularly pharmaceutical inspectors, who frequently submit samples should be provided with standard "test request" forms and such a form should accompany each sample submitted to the laboratory. It should provide the following information:

— the name of the institution or inspector that supplied the sample;

— the source of the material;
— a full description of the product, including its composition, brand name, dosage form, concentration or strength, manufacturer, and batch number (if available);
— the size of the sample, and the reason for requesting the analysis.

Other information that is often needed includes the date on which the sample was collected, the size of the consignment from which it was taken, the expiry date, and the pharmacopoeial specification to be used for testing.

When the sample is first received it should be immediately inspected to ensure that the labelling is in conformity with the information contained in the test request. If discrepancies are found, or if the sample is obviously damaged, the fact should be recorded at once on the test request form.

No sample should be examined until the relevant test request has been received. If this is lacking, the sample should be safely stored until all the relevant documentation has been received. In emergencies a request for analysis may be accepted verbally. In this event all details should immediately be placed on record pending the receipt of written confirmation.

Incoming samples and test requests should be numbered consecutively. For each sample a self-adhesive label bearing this registration number should be affixed to the container in such a way as not to obliterate other markings or inscriptions. If a request refers to two or more drugs, to different dosage forms, or to different batches of the same drug, separate registration numbers should be assigned to each. Photocopies of all documentation should accompany each numbered sample when it is forwarded to the lead unit.

2.4 Analytical worksheet

A printed analytical worksheet with space for the following information should be used by the analyst to confirm that the sample has been examined in accordance with the requirements and, when necessary, to provide documentary evidence to support regulatory action:

— the registration number of the sample;
— the date of the test request;
— a description of the sample received;
— the quality specifications to which the sample was tested (including any additional or special methods employed);
— the results obtained, including any calculations necessary;
— the interpretation of the results and final conclusions.

Additional space should be provided to indicate whether and when portions of the sample were forwarded to other units for special tests (e.g. sterility, infrared spectrum), and the date when the results were received. To ease the flow of information between collaborating units a further set of printed forms can be useful. These can be sent out in duplicate from the lead unit with the sample to which they refer. In due course one copy is returned to the lead unit for attachment to the analytical worksheet, while the other is retained in the unit that undertook the work.

A separate analytical worksheet should be completed for each numbered sample. Each completed worksheet should be signed by the analyst responsible, initialled by the supervisor and placed on file for safe-keeping together with any attachments, including calculations and tracings of instrumental analyses. If this information is filed centrally in a registry, a copy of the worksheet should be retained in the lead unit for ease of reference.

It is still the custom in many laboratories for each analyst to keep a complete record of his work in a bound laboratory notebook with numbered pages. Although this has value it is an inconvenient form of documentation in a modern laboratory where results obtained on recording instruments or printed calculations have to be entered into the worksheet. If such a notebook is kept, it should be regarded as a supporting record only.

On the day the sample is received in the unit, the registration number, the date, the name of the product, and a description of the material received should be entered on the analytical worksheet. The information contained in the test request should be checked against the data on the label and the findings recorded, dated, and initialled. Any discrepancies in the documentation, or between the data provided and the appearance of the sample, should also be recorded. Any queries should immediately be referred back to the provider of the sample.

The analyst must then determine what specification is to be used to assess the sample. In many cases, the test request will specify a particular pharmacopoeial monograph or manufacturer's specification and the analyst must find out whether the current version is available. If no precise instructions are given, the specification in the officially recognized national pharmacopoeia should be used or, failing that, the manufacturer's officially approved or other nationally recognized specification. The reference number of the specification should be entered on the worksheet and a photocopy of the document attached.

If no formally approved specification exists, preference should be given to a current monograph in a foreign pharmacopoeia. If no suitable pharmacopoeial monograph can be found, the requirements should be drafted in the laboratory itself on the basis of published information and any other relevant documentation (*1, 2*). Otherwise, if the general policy of the laboratory permits, the specification contained in the product licence may be requested from the manufacturer. Whatever happens, detailed notes on the specification selected and the methods of assessment used must be entered in the worksheet.

2.5 Testing

If specific tests such as sterility tests, pyrogen tests, or special physicochemical tests need to be carried out by another unit or by a specialized external laboratory, the analyst should prepare the request and arrange for the transfer of the required number of units (bottles, vials, tablets) from the sample. Each of these units should bear the correct registration number.

Testing should be started as soon as possible after the preliminary procedures have been completed. If this is not feasible, the reasons should be noted in the worksheet and the sample placed in a special locked storage cabinet.

Detailed guidance on test methods is contained in the general notices and monographs of official pharmacopoeias. The following principles therefore apply only when no pharmacopoeial requirements are available or when ambiguous results are obtained.

Provided the result is unequivocally positive and the analyst is well acquainted with the technique, replicate chemical and physicochemical tests are not, in general, required for identity tests if based upon colour reactions, precipitation tests, infrared spectra, ultraviolet identification, or thin-layer chromatography, nor are they required for purity tests based on the matching of colour or opacity against standards or on thin-layer chromatography. In some laboratories, however, purity tests are routinely run in duplicate as a check against accidental contamination. Assays to assess strength or level of impurity should always be replicated, however, whether they are based on titrimetry, gravimetry, colorimetry, ultraviolet measurements, gas-liquid chromatography, or high performance liquid chromatography. Replicate measurements should also be made of physical properties such as pH values, optical rotations, refractive indices, and melting temperatures. Whenever replicate measurements are made, the results should be recorded as the arithmetic mean of the estimates.

In other cases, the required number of replicate measurements is defined in the description of the method. This applies to physicochemical tests involving gas-liquid chromatography or high performance liquid chromatography and to biological assays whose results require statistical evaluation.

Whenever ambiguous results are obtained, or when the discrepancies between replicate measurements fall outside acceptable limits, at least two further replicate tests should be run, preferably by a different analyst. Any important discrepancies must be investigated. Aberrant results can be rejected only when they are clearly due to error. Otherwise, the mean values obtained by each analyst should be quoted separately to provide clear confirmation that the sample failed the test.

Errors arise not only because of human failings but also as a result of unsuitable or deteriorated reagents and chemical reference substances, inadequate instrumentation, inappropriate methods (particularly methods that are difficult to reproduce), and variations in the laboratory environment. Comparative estimations on standard samples can frequently help to detect such errors, particularly in cases in which the analyst lacks experience in the method he has used.

All values obtained in each test, including blank results, should immediately be entered on the worksheet, and all graphical data, whether obtained from recording instruments or hand-plotted, should be attached.

2.6 Evaluation of test results

The analyst should review the results as soon as possible after all the tests have been completed to determine whether they are mutually consistent and whether they meet the specification. All conclusions should be entered on the worksheet by the analyst and initialled by the supervisor.

The certificate of analysis issued by the laboratory should be based on the analytical worksheet. It should specify the sample and the registration number, state the specification to which the sample was tested, list and provide the results of all the tests that were performed and state whether or not the sample was found to comply with the requirements. Certificates stating that a sample is not in compliance with the required specification must always be signed by the head of the laboratory.

A sample may be recorded on the worksheet as conforming to specification only if it meets all the relevant requirements. Any discrepancy confirmed by replicate testing should be evaluated in relation to the results of the other tests and the conclusions reached should be discussed with the head of the laboratory before they are entered on the worksheet. This record should then be signed by each of the analysts involved.

In large laboratories responsibility for certifying samples that conform to specification usually lies with the lead unit. However, in the event of non-compliance, the head of the laboratory is ultimately responsible for recommending any regulatory action that is required.

2.7 Retention samples

A retention sample originating from the same consignment as the analytical sample must always be kept in the laboratory—when possible in the original container—for use if the results of the analysis are disputed. This is usually prepared by the lead unit from the sample as received. The sample should therefore be large enough to provide an adequate reserve even when a number of replicate tests are required.

Sometimes, however, the retention sample is prepared by the sampling inspector when the analytical sample is taken. In this case the two samples should be separately packaged and transferred together to the laboratory. The retention sample is then labelled as such and given a registration number before it is forwarded with the analytical sample for storage in the lead unit.

Once all the required tests have been performed, any remaining portions of the sample should be resealed in their original containers. They should then be labelled with the date on which they may be discarded and placed in a locked cabinet in central store, if necessary at low temperature. Samples found to comply with specification should be kept for at least 6 months. Those that do not should be kept for at least one year, or for any longer period specified in current regulations.

2.8 Specifications repertory

Every drug control laboratory must possess the current versions of all the specifications that it needs, whether they are contained in pharmacopoeial compendia or in manufacturers' registration documents. In a large laboratory the specifications repertory is a documentation service with responsibility for updating all the pharmacopoeias—including supplements, addenda, and corrections—used in the laboratory and maintaining a specifications file for all drugs marketed within the country.

The repertory should retain a list of all pharmacopoeias in the laboratory and ensure that adequate numbers of supplements and addenda are ordered. All updatings and corrections should be noted in the principal volumes to prevent obsolete sections being used. Additional or replacement pages for loose-leaf publications should be inserted immediately they are received; pages no longer valid should be removed.

In addition, every laboratory should maintain a file of non-pharmacopoeial quality specifications for drugs tested to specifications established either by the manufacturer or by the laboratory itself. The range of monographs in this file will depend on current legal requirements and on whether or not a published national or regional pharmacopoeia is accorded official status within the country. Each entry should be numbered and dated so that the latest revision can easily be seen. The copy in the repertory file should bear the date of approval by the national registration authority or the lead unit and any other information relevant to the status of the monograph. All subsequent corrections or changes should be entered in these copies and endorsed with the date and the initials of the person making the entry. The master copy should never be released from the repertory; for laboratory use photocopies should be taken.

Manufacturer's specifications are the property of the company and in some countries are made available to governments strictly for registration purposes. In this case the quality control laboratory may need to negotiate their release with manufacturers or even, in some cases, to develop independent specifications. In other countries national laboratories are routinely asked to give their opinion on the specifications for each newly introduced product when it is registered by the drug regulatory authority.

2.9 Reagents

All reagents, including solvents, used in tests and assays must be of appropriate quality. They should be purchased from reputable manufacturers or dealers, preferably in small factory-filled containers suitable for laboratory use. Stocks stored in greater bulk are more vulnerable to contamination and degradation. Appropriate safety regulations should be drawn up and rigorously implemented wherever toxic or flammable reagents are stored or used. Those subject to poison regulations or to the controls applied to narcotic and psychotropic

substances should be clearly marked as "Poison" and kept separately from other reagents in locked cabinets. A register of these substances must be maintained by the responsible member of staff. The head of each unit must accept personal responsibility for the safe-keeping of any of these reagents kept in the workplace.

Reagents made up in the laboratory should be prepared according to prescribed procedures and, when applicable, to published pharmacopoeial or other standards. Each label should clearly specify the contents, the manufacturer, the date received, and, as appropriate, the concentration, standardization factor, shelf-life, and storage conditions. Volumetric solutions made up by dilution should be labelled with the name of the manufacturer of the concentrate, the date of preparation, and the initials of the responsible technician.

Responsibility for making up reagents in the laboratory should be clearly assigned. Standardization of procedures is more readily implemented when this work is supervised by one person, even when the same reagents are used in several units. However, the reagents should not be moved unnecessarily from unit to unit and should be transported, whenever possible, in their original containers. When they are subdivided, they should always be transferred into scrupulously clean, fully labelled containers.

Whatever routine precautions are taken to ensure the adequacy of volumetric solutions, they should be checked whenever they are used in a test which indicates that a sample is not in compliance with specifications and the results of the check should be attached to the analytical worksheet.

Distilled water and deionized water should also be regarded as reagents and precautions should be taken to avoid contamination during their supply and distribution. Stocks should be checked at least once a month to ensure that they meet quality requirements: the specific conductance at 20 °C should not be greater than 2.0×10^{-6} ohm^{-1} cm^{-1} and the chloride ion content should meet current pharmacopoeial requirements for purified water.

All reagent containers should be inspected to ensure that seals are intact both when they are delivered to the reagent store and when they are distributed to the units. These inspections should be recorded by initialling and dating the labels. Reagents that appear to have been tampered with should be rejected except in rare instances when their identity and purity can be confirmed by testing. Maintaining stocks of reagents in a central store promotes safety and continuity of supplies, particularly for substances that need to be ordered long in advance of delivery.

In a large laboratory the storage area should provide separate rooms for flammable substances, for fuming acids, including concentrated hydrochloric acid, nitric acid, and bromine, and for ammonia and volatile amines. Self-igniting materials, such as metallic sodium and potassium, should also be stored separately. All storage areas should be located and equipped in accordance with fire regulations. To promote safety and to reduce contamination of the laboratory environment, these reagents should never be stored elsewhere in the laboratory without good reason.

The store should be kept stocked up with the clean bottles, vials, spoons, funnels, and self-adhesive labels required for dispensing reagents from larger to smaller containers. Special equipment may be needed for the transfer of larger volumes of corrosive liquids. The storekeeper should be trained to handle chemicals with the necessary care and safety.

2.10 Reference materials

Details of all the reference materials required should be kept in a central register. In a large laboratory this responsibility should be assigned to a specific person designated as the reference materia. coordinator. A national drug control laboratory that is required to establish reference materials for other institutions or for drug manufacturers will need to create a separate reference materials unit which will assume all the duties of the coordinator.

The register should contain details not only of all official reference substances and reference preparations, but also of secondary reference materials and non-official materials prepared in the laboratory as working standards. Each entry should be assigned a number and should give a precise description of the material, its source, the date of receipt, the batch designation or other identifying code, the intended use of the material (infrared reference material, impurity reference material for thin-layer chromatography, etc.), the place in the laboratory where it is stored, and any special storage conditions.

In addition to the register, a file should be kept containing full information on the properties of each reference material. In the case of working standards prepared in the laboratory the file should include the results of all tests and checks used to establish the standard and the initials of the responsible analyst.

Its laboratory identification number should be marked on each vial of the material and this must be quoted in the analytical worksheet every time it is used. A new number should be assigned to each new batch of material as soon as it is delivered or prepared. All reference materials should be inspected at regular intervals to make sure that they have not deteriorated and that they are being stored under appropriate conditions.

Further guidance on establishing, handling, and storing reference materials is contained in Annex 1 of the twenty-eighth report of this Committee (*3*).

2.11 Instruments and their calibration

Instruments are subject to wear, corrosion, and mishandling. If they are not in good working order they may give rise to serious analytical errors that may remain undetected unless systematic checks are made.

Whenever possible, regular servicing of instruments by specialist maintenance teams should be arranged. Instruments exposed to high levels of humidity should be resistant to corrosion and adequately protected against mould and fungal growth. Where line voltage is variable, suitable voltage stabilizers should be installed.

Some instruments may need to be protected from extremes of humidity or temperature in a specially designed area. Otherwise, analytical instruments can be either grouped together or dispersed between the various units. The choice will depend on the types of instruments, their fragility, the extent to which they are used, and the skills required to operate them.

Regular calibration of all instruments used to measure the physical properties of substances is essential and specific schedules should be established for each type of instrument, having regard to the extent to which it is used. pH meters should be calibrated at least once a day. The reliability of the wavelength scale of melting-point instruments and spectrophotometers operating in the ultraviolet region should be checked once a week and a full calibration undertaken once a month. Infrared spectrophotometers require calibration every quarter, while refractometers and spectrofluorometers should be serviced half-yearly. Analytical balances should also be serviced at least half-yearly by a qualified balance specialist.

Volume 1 of the third edition of *The international pharmacopoeia* describes the procedure for calibrating refractometers, thermometers used for the determination of melting temperature, and potentiometers for pH determination (*4*). It also explains the methods for checking the reliability of the scales on ultraviolet and infrared spectrophotometers and spectrofluorometers. A clear description of the standard operating procedure should be placed beside each instrument together with a schedule of the dates on which it is due for calibration.

Whatever routine precautions are taken to ensure the calibration of instruments, they should also be checked whenever they are used in a test which indicates that a sample is not in compliance with specification. The results of the check should be attached to the analytical worksheet.

2.12 Safety in drug control laboratories

Safety depends on the maintenance of exemplary technical standards and laboratory discipline. Safety instructions, both general and specific, should be given to each new member of staff and should be regularly supplemented with written material, poster displays, audio-visual material, and occasional seminars.

General rules for safe working include:

(1) prohibition of smoking, eating, and drinking in the laboratory;
(2) familiarity with the use of fire-fighting equipment, including fire extinguishers, fire blankets, and gas masks;
(3) use of laboratory coats or other protective clothing;
(4) adequate insulation and spark-proofing of electrical wiring and equipment, including refrigerators;
(5) full labelling of all containers of chemicals, including prominent warnings (e.g., "Poison", "Flammable") whenever appropriate;
(6) observation of safety rules in handling cylinders of compressed gases and familiarity with their colour identification codes;
(7) avoidance of solitary work in the laboratory;
(8) provision of first-aid materials and instruction in first-aid techniques, emergency care, and use of antidotes.

Protective clothing should be available, including goggles, masks, and gloves. Rubber suction bulbs should be used on all pipettes and siphons. Staff should be instructed in the safe handling of glassware, corrosive reagents, and solvents, and particularly in the use of safety containers or baskets to avoid spillage from containers. They should also be warned of the danger of violent, uncontrollable or dangerous reactions when mixing specific reagents. They must be instructed in the precautions required when, for example, mixing water and acids, acetone-chloroform and ammonia, or flammable products and oxidizing agents, and they should avoid the use of peroxidized solvents. They must also be instructed in the safe disposal of unwanted corrosive or dangerous products by neutralization or deactivation and of the need for safe and complete disposal of mercury and its salts.

While particularly poisonous or hazardous products must be singled out and appropriately labelled, **it should not be taken for granted that all other chemicals are safe**. All unnecessary contact with reagents, especially with solvents and their vapours, should be avoided. The use of known carcinogens and mutagens should be limited or totally excluded if required by local regulations. Replacement of toxic solvents and reagents by less toxic materials should always be the aim, particularly when new techniques are developed.

References

1. WHO Technical Report Series, No. 645, 1980, Annex 2.

2. WHO Technical Report Series, No. 614, 1977, Annex 1.

3. WHO Technical Report Series, No. 681, 1982, Annex 1.

4. *The international pharmacopoeia*, third edition, volume 1: *General methods of analysis*. Geneva, World Health Organization, 1979.

PRACTICAL TRAINING

STANDARDISATION & QUALITY CONTROL OF MEDICINAL & AROMATIC PLANTS & THEIR PRODUCTS

Determination of Essential oils in Citronella oil

Citronella Oil is obtained by distillation from *Cymbopogon nardus* Rendle or *C. winterianus* Jowit(Fam. Gramineae) or from varietal or hybrid forms of these species;producing countries include Sri Lanka,Indonesia,Taiwan,Thailand and other tropical countries.

Morphology

Perennial herb up to 2 m high, with rhizome . Basal leaf sheaths persistent and revolute; blades up to 1 m long, 5-20 mm wide. Racemes in pair, with spathose involucre in base, then arranged in dense large paniclel spikelets opposite on each node; fertile ones sessile,ovoid-lanceolate, about 3 mm long ; first glume 2-lobed, slightly flat in dorsal surface, veins inconspicuous, narrow-winged; second lemma linear,awnec.

Uses

The essential oil is widely used.Geraniol and citronellol extracted from it are important raw materials for mixing various cosmetics and soap essences. Citronellal is also used as insect repellent including mosquito repellent.

Constituent

The herb contains 0.37-0.40% of essential oils. Chemical constituents of the oil are as follows.

- Alpha-pinene -Camphene -Beta-pinene
- Limonene -Linalool -Iso-pulegol
- Citronellal -Citronellol -Beta-citral
- Geraniol -Alpha-citral -Citranellyl acetate
- Eugenol -Geranyl acetate -Beta-Bourbonene
- Beta-elemene -Beta-caryophyllene -Gamma-cadinene
- Beta-cubebene -Alpha-muurolene -Gamma-muurolene
- Delta-cadinene -guaiene

We are now able to ng the method of determination the essential oils in Citronella oil by utilizing Gas Chromatography with Mass Spectrometry (GC-MS). The chromatographic condition and the sample preparation are described as follows:

Sample preparation

1. Transfer sample 30 µl into a 10- ml volumetric flask
2. Sonicate until completely dissolved
3. Inject 1.0 µl of the above solution to the GC-MS

Chromatographic Conditions

Column = PE-5 MS

Length = 20 m

Carrier gas = Helium 99.999%

Flow rate = 0.5 ml/min

Split ratio = 10:1

Oven temperature = 75 C,hold 3 min ———200 C, rate 4C/min,hold 5 min

Inj. Temperature = 270 C

Detector (MS)

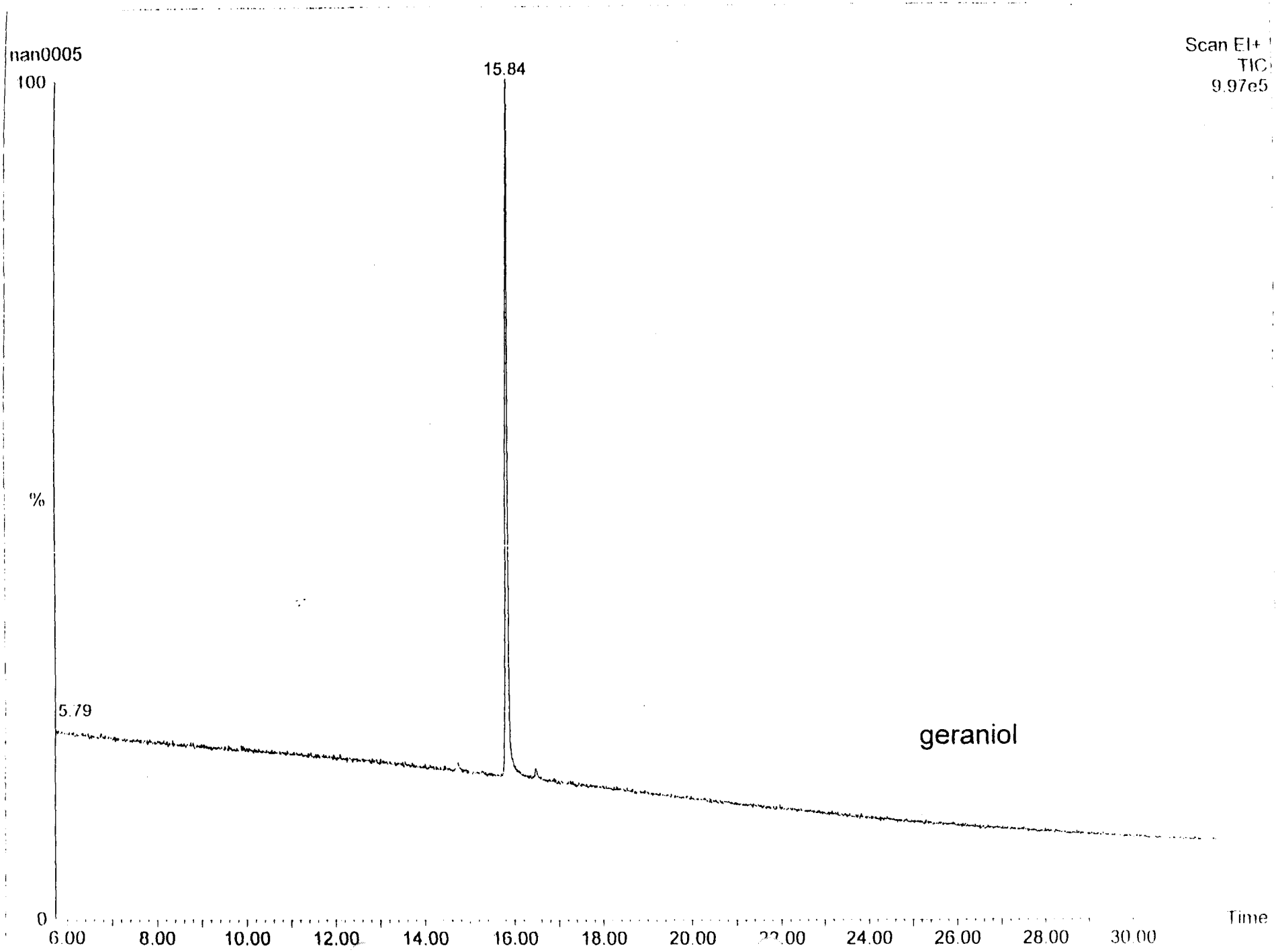
Ion source temp. = 220 C

Heater = 250 C

Total Run time =44.25 min

Reference

1. Quality control methods for medicinal plant materials. World Health Organization Geneva,p.47-60
2. Dr. Brian M.Lawrence.Progress in Essential Oils. Essential Oils b22,1981-1987,p.29,147,1981-1987
3. British Pharmaceutical Codex,115,1973
4. The Merck Index 11,p.362-364,1989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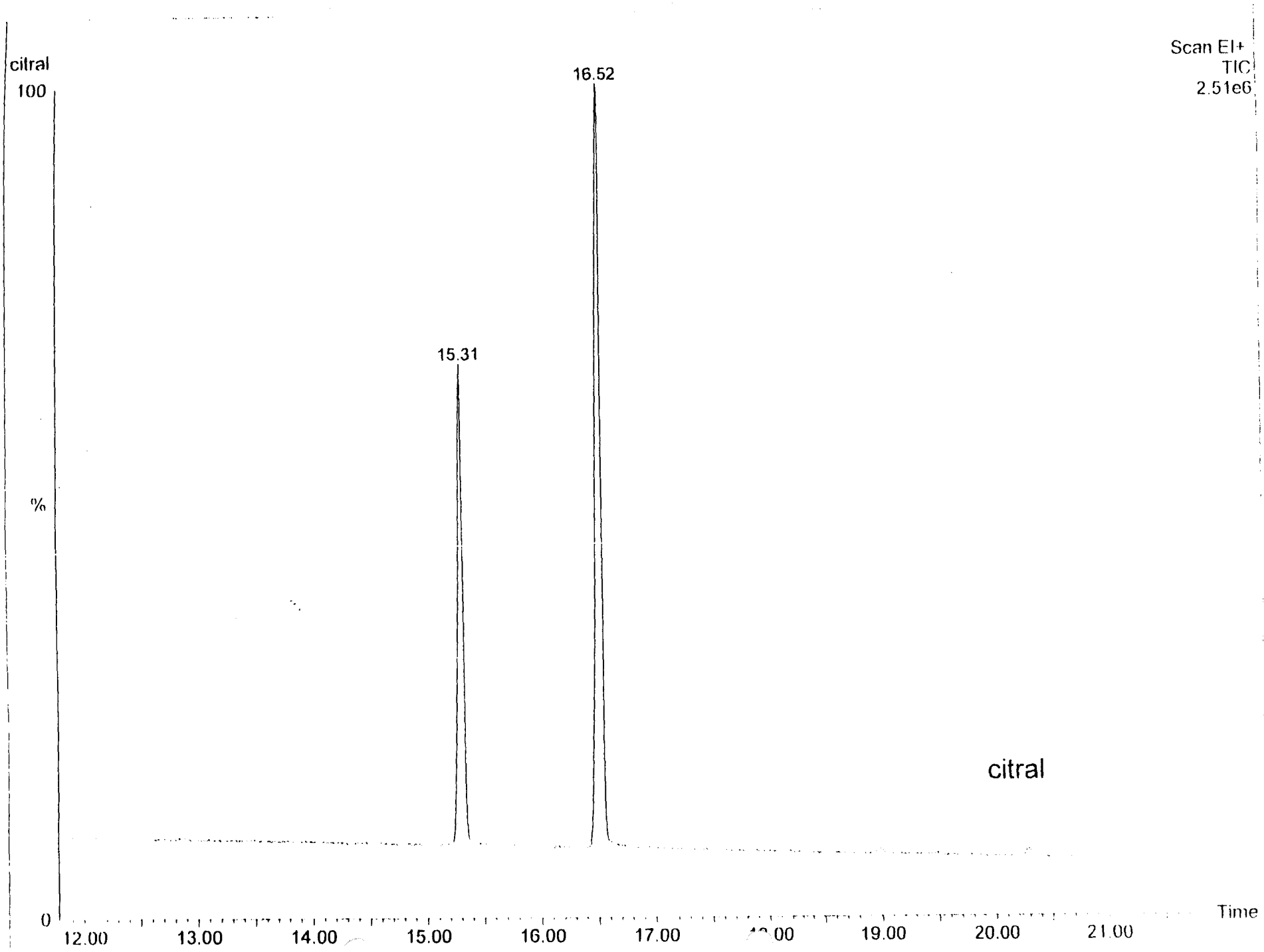
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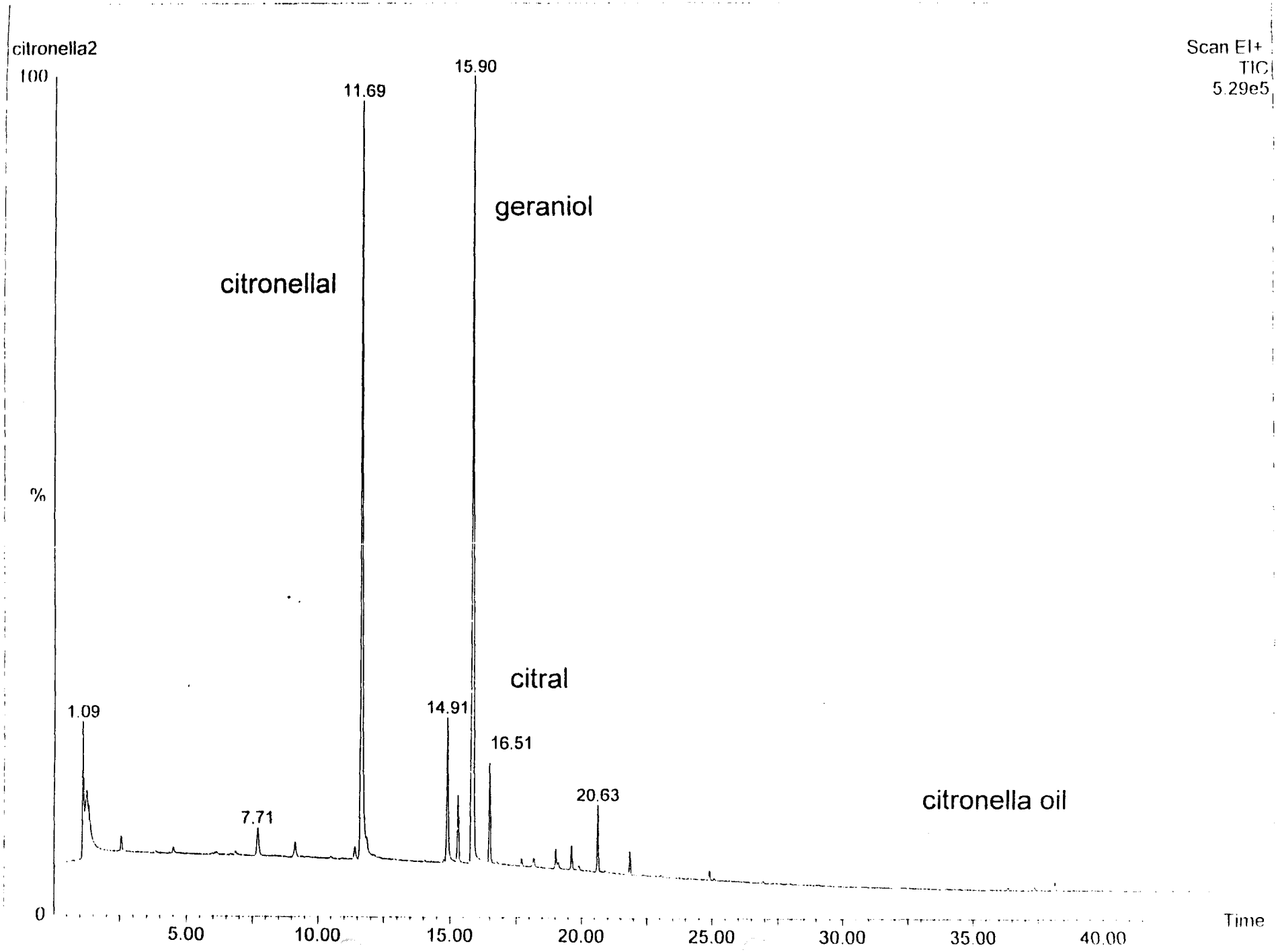
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Time

A. References

Yaowu-Fenxi-Zazhi. Nov 1995; 15(16) : 36-39

Thai Herbal Pharmacopoeia 1995, Vol. I, p. 22.

B. Definition

AR grade	:	Analytical reagent grade

C. Specification

Description	:	Dark green powder with characteristic odour
% Content of Rhein	:	Not less than 4.5 mg % w/w
% Water content	:	Not more than 11.0 % w/w

D. Equipment and reagent

1. Camag® TLC Scanner II (Refer to SOP No. PR-06-016)
2. Camag® Evaluation software (Refer to SOP No. PR-06-016)
3. Camag® automatic TLC Sample III (Refer to SOP No. PR-06-016)
4. Rhein standard
5. Methanol (AR grade)

E. Procedure

Preparation of Rhein Standard Solution

1. Accurately weigh 3 mg of Rhein standard into a 10-ml volumetric flask.
2. Dissolve and adjust the volume with methanol to obtain the 0.3 mg/ml standard solution.
3. Pipette 1 ml of this solution into a 10-ml volumetric flask and adjust volume with methanol to obtain the 0.03 mg/ml standard solution.
4. Filter the solution through a 0.45 µm membrane filter and spray the filtrate with HPTLC system.

Preparation of *Cassia alata* leaves solution

1. Accurately weight 1 g of dry pulurized leaves of *Cassia alata* into a 25-ml erlenmeyer flask.
2. Add 20 ml of methanol and sonicate for 15 min.
3. Filter the solution through filter paper.
4. Evaporate this solution and adjust to 25 ml with methanol.
5. Filter the solution through a membrane filter and collect the filtracte to spray with HPTLC system.

F. Assay procedure

Apply 10,000 nl each of Test solution and 2,000, 3,000, 4,000, 5,000 and 6,000 nl of standard solution in different tracks on a precoated Kieselgel 60 F_{254} plate (10 x 10 cm) of uniform thickness (0.2 mm). Develop the plate in the solvent system.

G. Cromatographic conditions

Plate	:	Kieselgel 60 F_{254}
Developing system	:	Petroleum ether : n-Hexane : Ethyl acetate : Acetic acid : Methanol 15 : 30 : 15 : 1 : 1
Application volume	:	Rhein standard 2,000, 3,000, 4,000, 5,000 and 6,000 nl Sample 10,000 nl
Detector	:	UV 435 nm

H. Calculation

1. Plot standard curve between the peak area (as y values) and the concentration (as x values) of the 5 standard solutions to obtain the following equation (1)

$$y = Bx + A \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where y = Peak area of Rhein component

x = Concentration of Rhein (mg/ml)

A = y-intercept

B = Slope

2. Use equation (1) to calculate the concentration of Rhein

3. Calculate percent weight of Rhein

$$\% \text{ Content of Rhein} = C_{Samp} \times 25 \times \frac{100}{Wt}$$

where C_{Samp} = Concentration of sample (mg/ml)

Wt = Actual weight of sample (g)

A. References

- USP23, General Chapter (1225), Validation of compendial method, P. 1982-1984

The following validation characteristics were determined :

Selectivity	Accuracy	Linearity
System suitability	Precision	Ruggedness

B. Equipment and Reagents

1. Pump : Spectra SYSTEM P1000 (Thermo Separation Products) (Refer to SOP No. PR-06-004)
2. Integrator : PC1000 (Refer to SOP No. PR-06-004)
3. Detector : Spectra SYSTEM UV1000 (Refer to SOP No. PR-06-004)
4. Autosampler : Spectra SYSTEM AS3000 (Refer to SOP No. PR-06-004)
5. Methanol HPLC grade
6. Zidovudine (internal standard)
7. Stavudine std
8. Distilled water

C. Chromatographic Condition

Column	:	Water Spherisorb® 5 µm ODS, 25 cm x 4.6 mm
Mobile phase	:	Methanol : Distilled water (40 : 60)
Flow rate	:	1.0 ml/min
Pressure	:	3000 Psi
Detector wavelength	:	UV 260 nm
Injection volume	:	20 µl

D. Selectivity

The selectivity of the method was determined by injecting Stavudine, Zidovudine and the placebo (containing Explotab, Microcrystalline cellulose and Magnesium stearate) onto the chromatographic system previously described.

The retention times of each compound were given below:

	Absolute retention time (min)
Stavudine	3.142
Zidovudine	4.986
Placebo	-

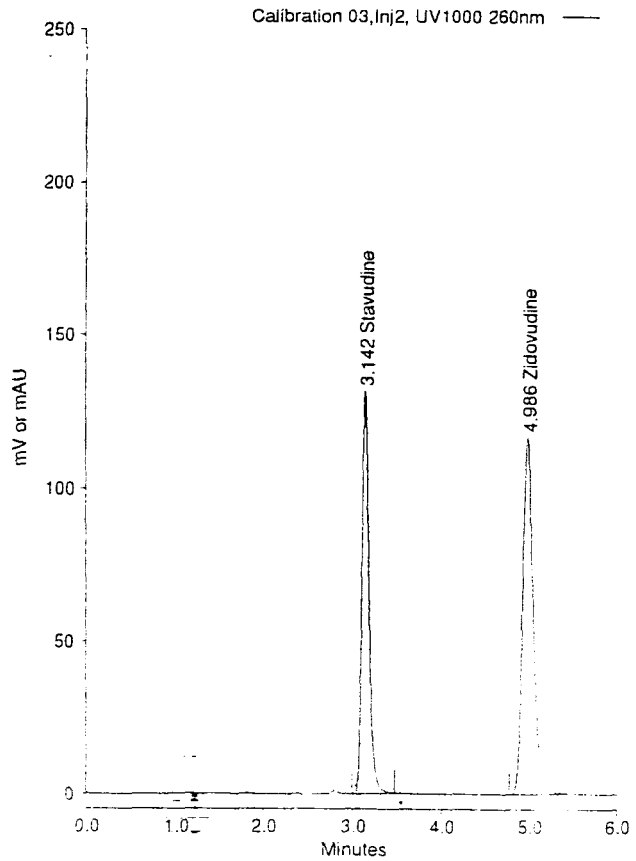


Figure 1 : Chromatogram of Stavudine and Zidovudine

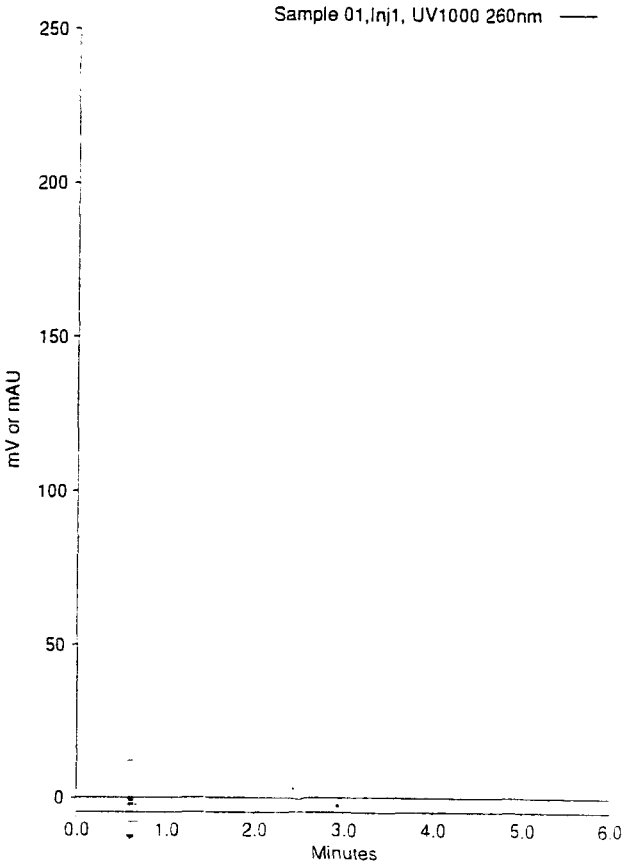


Figure 2 : Chromatogram of Additive

From the chromatogram obtained, it is concluded that the proposed method is specific to analyse stavudine in pharmaceutical dosage form (capsule).

E. Accuracy

The accuracy of method was tested via recovery of reference standard spiked in Sample (standard addition method)

Sample preparation are described below :

1. Weigh and mix powder 20 capsules
2. Transfer an accurately weighed portion of the powder, about 210 mg of stavudine powder to a 50-ml volumetric flask
3. Add about 30 ml of distilled water, sonicate for 15 minutes

4. Adjust to volume with distilled water and centrifuge this solution at 3000 rpm for 10 minutes

5. Pipet 2.0 ml of supernatant into a 50-ml volumetric flask and adjust to volume with distilled water (stock sample)

6. Transfer 1.0 ml of stock sample into six separate 25-ml volumetric flasks.

7. Add variable amount of standard stavudine (duplicate) into each flasks to obtain concentrations of 0, 0.005, 0.01, 0.015, 0.020, 0.025 mg/ml, respectively.

8. Add 1 ml of internal (Zidovudine conc. 0.025 mg/ml in distilled water) into the volumetric flasks.

9. Adjust to volume with distilled water and mix.

The solutions were then analysed according to the above chromatographic condition. The percent recoveries of the active were calculated.

Stavudine added per ml (mg)	Stavudine found per ml (mg)	Recovery (%)
0.00484	0.00486	100.50
0.00968	0.00962	99.44
0.01452	0.01459	100.52
0.01935	0.01913	98.83
0.02419	0.02395	99.00

$\bar{X}$ = 99.66%

RSD = 0.81%

95% confidence interval = 98.65-100.66

F. Precision

1. Repeatability

The content of Stavudine was determined by assaying 6 different samples of Stavudine capsule taken from Pharmacy section Id. No. 125/41 in accordance with the below procedure with-in a day using the same equipment.

Procedure :

- Weigh and mix powder 20 capsules
- Transfer an accurately weighed portion of the powder, about 210 mg of stavudine powder to a 50-ml volumetric flask
- Add about 30 ml of distilled water, sonicate for 15 minutes
- Adjust to volume with distilled water and centrifuge this solution at 3000 rpm for 10 minutes
- Pipet 1.0 ml of supernatant into a 50-ml volumetric flask and adjust to volume with distilled water
- Add 2.0 ml of internal standard (Zidovudine conc. 0.025 mg/ml in distilled water) into volumetric flask
- Adjust to volume with distilled water and mix

Trial No.	Stavudine (% LA)
1	102.89
2	103.34
3	103.10
4	103.38
5	102.83
6	104.46

$\bar{X}$ = 103.33

SD = 0.60

%RSD = 0.58

2. Reproducibility

The content of stavudine was determined by assaying 6 different samples of stavudine capsule from Pharmacy section Id. No. 125/41 in accordance with the analytical procedure on different days using the same analyst and the same equipment.

	Day 1	Day 2
	102.89	98.82
	103.34	100.55
	103.10	97.61
	103.38	98.50
	102.83	99.05
	104.46	97.67
$\bar{X}$	103.33	98.70
SD	0.60	1.08
%RSD	0.58	1.10
95% confidence interval	102.65-104.01	97.48-99.92

% RSD between days = 2.53

G. Linearity

1. Linearity of system

Prepare five standard solutions having final concentrations of 0.005, 0.010, 0.015, 0.020 and 0.025 mg/ml. The concentration of the internal standard, zidovudine, was constant in all standard solutions (0.025 mg/ml). The linear regression curve is illustrated as below :

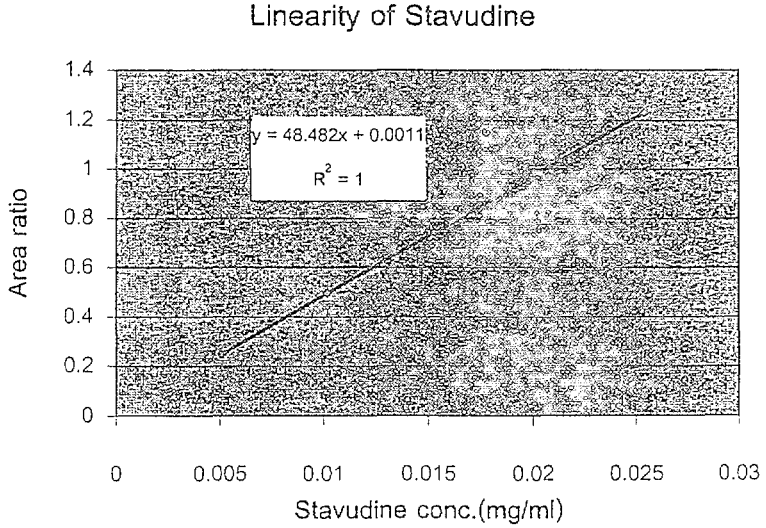


Figure 3 : Linearity of system

By least squares analysis of the data, the equation of the line is y = ax+b, where "y" is the peak area ratio and "x" is the concentration of stavudine in mg/ml

a = 48.482, b = 0.0011, Correlation coefficient = 1.0000

Due to the linear correlation coefficient is 1 assumed that the model is linear in the concentration range studied (0.005-0.025 mg/ml)

2. Linearity of method

The linearity of method was performed by plotting the amounts of stavudine added against the amounts of stavudine found (the values obtained from section E).

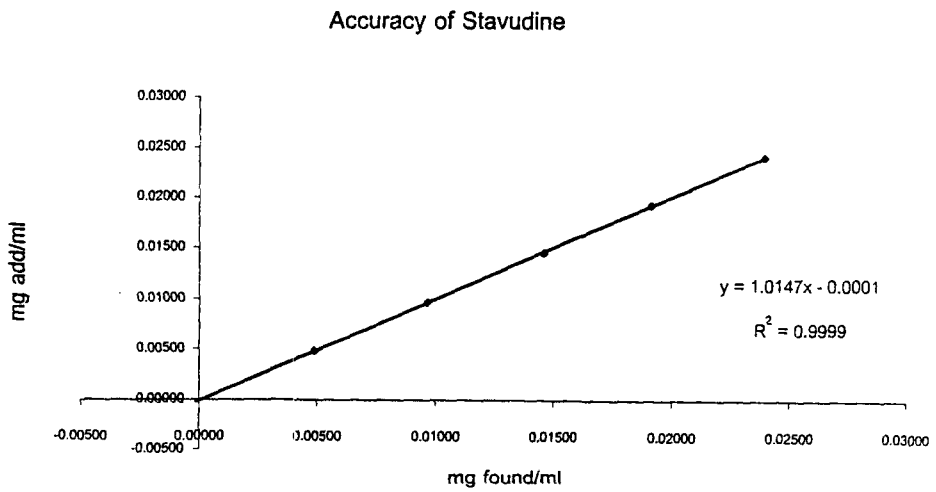


Figure 4 : Linearity of method

RDI QUALITY CONTROL LABORATORY	METHOD NO. VT-C013
	REV. NO. 1.0
TITLE : Validation of Stavudine capsule 30 mg	PAGE 10 OF 12

H. Ruggedness

The ruggedness of the analytical method was determined by analysing 6 samples of stavudine capsule from Pharmacy section Id. No. 125/41 using different analysts and using different instruments.

1. Analyst

	Analyst 1	Analyst 2
	102.97	107.14
	102.41	106.66
	103.64	106.99
	100.86	108.91
	102.52	108.16
	103.45	106.98
$\bar{X}$	102.64	107.47
SD	1.00	0.87
%RSD	0.97	0.81
95% confidence interval	101.51-103.77	106.48-108.46

% RSD between days = 2.55

2. Instrument

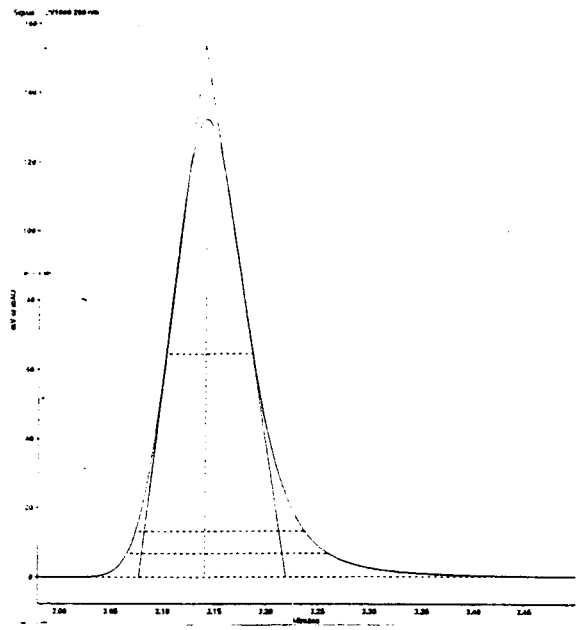
	Instrument 1	Instrument 2
	102.89	102.97
	103.34	102.41
	103.10	103.64
	103.38	100.86
	102.83	102.52
	104.46	103.45
$\bar{X}$	103.33	102.64
SD	0.60	1.00
%RSD	0.58	0.97
95% confidence interval	102.65-104.01	101.51-103.77

% RSD between days = 0.839

RDI QUALITY CONTROL LABORATORY	METHOD NO. VT-C013
	REV. NO. 1.0
TITLE : Validation of Stavudine capsule 30 mg	PAGE 11 OF 12

I. System suitability of the analytical method

- Tailing factor (USP) = 1.2862 (Stavudine)
  = 1.2194 (Zidovudine)
- Resolution factor (USP) = 10.1



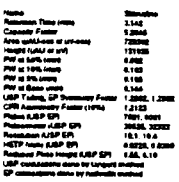
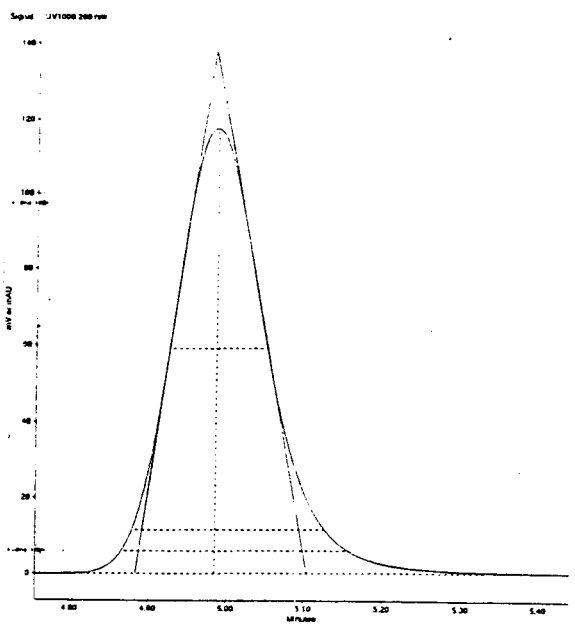


Figure 5 : Stavudine peak



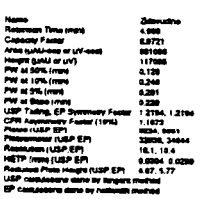


Figure 6 : Zidovudine peak

- Repeatability of peak areas

Repeatability of peak areas was determined by injecting a standard solution 6 times. The relative standard deviation of the peak responses was measured as the peak area ratio USP prescribes a relative standard deviation of less 2% in case of 5 injections.

Trial No.	Peak area ratio
1	0.74281
2	0.74345
3	0.74255
4	0.74273
5	0.74257
6	0.74284

$\bar{X}$ = 0.74282

SD = 0.00033

%RSD = 0.04416

J. Chromatogram

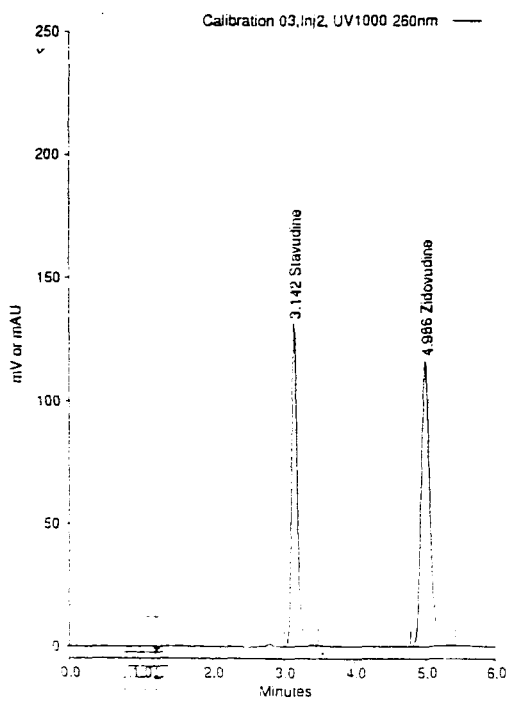


Figure 7 : Chromatogram of standard solution

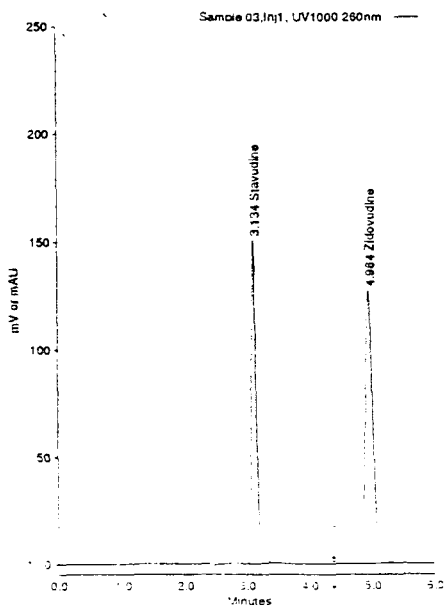


Figure 8 : Chromatogram of sample solution

DOCUMENTATION METHODS FOR PRESERVING ETHNOMEDICAL KNOWLEDGE

Professor S. S. Handa

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname :_____Handa_____Other name :_____S.S._____

Title (Mr, Mrs, Dr, Professor, etc) _____Professor_____

Current Position :_____Chief Consultant R&D Zandu Pharmaceuticals Mumbai_____

Current Place of Work :____Zandu Pharmaceutical Works, Mumbai_____

Educational profile:

Year	Place of Study	Qualification	Field of study
1975	London School of Pharmacy	Ph.D.	Pharmacognosy

Professional Experiences :

___-Fellow of National Academy of Sciences _____

___-Fellow of National Academy of Indian Medicine_____

___-30 Years Teaching Pharmaceutical Sciences & Research at Paryab University_____

____Institute of Pharmaceutical Sciences_____

___-Chaudigarh & Later as Director Regional Research Laboratory (CSIR)_____

___-Recipient of Ranbaxy Research Award in Pharmaceutical Sciences_____

_____

Current interests :

___-Research & Development in Herbal Drug Industry_____

___-Chairman Medicinal Plant Expert Group of Indian_____

___-Associated with Ayurvedic Pharmacopoeia of India_____

___-Indian Pharmacopoeia and National Board of Bioresource Development_____

_____

_____

DOCUMENTATION METHODS FOR PRESERVING ETHNOMEDICAL KNOWLEDGE

Professor S. S. Handa
Chief Consultant R & D
Zandu Pharmaceutical Works Ltd.,
Mumbai, India

Lecture at a Training Course on

"Research Strategies on Medicinal and Aromatic Plants"

Organized By

International Centre for Science and High Technology - UNIDO

In Collaboration with

Research and Development Institute
Government Pharmaceutical Organization

Bangkok, Thailand
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CONTENTS

ABSTRACT

It was realised towards the end of twentieth century, more than ever before, that the biological richness and diversity of any country constitutes a repertoire of wealth waiting to be tapped. This revelation, shaped by new developments in the international trade regulations and the property rights over the biological resources and ethnomedical knowledge have particularly offered an hitherto unrealised potentiality for some of the developing countries which harbour much of the global biological diversity. Obviously there is a need to awaken to this truth and prepare ourselves in spheres such as legal protection through this wealth, conserving it from being over exploited and developing technical skills and information base to realize their full potentiality. In the wake of explosion of information on the medicinal plant wealth and their uses, it is important for each country to develop documentation system, which can protect country's ethnomedical knowledge and biological wealth. The present paper covers information on traditional medical knowledge in India, indigenous perspective, gathering / documenting ethnomedical information, indexing & abstracting of information, need for establishing database on ethnomedical knowledge and sources of information on medicinal plants.

Documentation Methods for Preserving Ethnomedical Knowledge

S. S. Handa

INTRODUCTION

Knowledge cannot be pigeonholed in watertight compartments. The evolution of knowledge systems has been continuous in its qualitative and quantitative dimensions but discontinuous in their method of preservation. Tribal and indigenous communities have generally preserved their traditions, art, culture and agriculture, although the present information and development age has tended to disrupt the continuity in their life style and sources of livelihood. The ecological and social problems of the unsustainable life styles and consumption pattern associated with modern civilization have resulted in a rekindling of interest in ethnobiology and indigenous knowledge systems. The need to conserve and reward indigenous knowledge systems has been articulated in the publication titled "Conserving indigenous knowledge integrating two systems of innovation" (UNDP 1994). There is no disagreement now on the need for recognising and rewarding informal innovation as related to conservation of plant and animal genetic resources. The question is how to do this. What is urgently needed is a transparent and easily implementable system of rewarding the contributions of tribal and rural women and men in the conservation and genetic enhancement of wild species and folk varieties. An important requirement for recognising and rewarding the intellectual contributions of tribal and rural families in genetic conservation and enhancement is the compilation of multimedia databases on the intellectual property rights of such families.

There is an urgent need to document, protect and propogate the traditional knowledge among the communities through various educational and awareness programmes as the new generation is lacking interest in continuing traditional practices based on traditional knowledge of medicinal plants. The protection of

indigenous knowledge and tradition and protecting intellectual property rights (IPR) to the benefit of the traditional medical practitioners such as vaidyas hakims siddhas local healers herbalists communities and individuals firms scientists etc require to be taken up as a national task. The protections to be provided for preventing the exploitation of the indigenous traditional knowledge for commercial purposes.

Traditional Medical Knowledge In India

The biodiversity of medicinal plants is associated with very rich cultural diversity related to India's traditional systems of medicine. Traditional medicine as practised in India consists of two streams, viz., folk medicine and the codified systems of medicine.

The folk medicine

This is a diverse stream, which is ecosystem and ethnic community specific. It is an oral tradition purely empirical in nature that exists in all rural communities throughout the length and breadth of India. There are millions of house- wives and elders who possess knowledge of home - remedies, food and nutrition related issues. Nearly 700,000 traditional birth attendants treat normal deliveries. Approximately 300,000 herbal healers treat common ailments. About 60,000 traditional bone setters treat orthopaedic problems. One comes across many examples of great depth and range of folk traditions in unpublished reports on medical practices in different regions. For instance in 1793, two medical officers of the East India Company - James Finlay and Thomas Cruso - reported on practice of rhinoplasty by a potter's community in Pune district in the Madras Gazette (and later in 1794 in the London Gentleman's Magazine). It was this technical report that led to further developments in Britian of plastic surgery of nose.

Codified Traditional medicines

Systems like Ayurveda, Unani, Siddha & Tibetan are expressions of this stream. The codified stream consists of medical knowledge with sophisticated theoretical foundations expressed in thousands of regional manuscripts covering treatises on

all branches of medicines and surgery. However of an estimated 100,000 medical manuscripts lying in oriental libraries and private collections in India & abroad, less than one percent are available & in current use by students and teachers in Indian medical schools. The earliest Ayurvedic texts, the *Sushruta Samhita* & *Charka Samhita*, are believed to have been written between 1500 & 1000 BC. There are at present over 400,000 licensed registered practitioners of the codified stream practising in towns & cities of India.

The Government of India has established an independent department of Indian Systems of Medicines under Ministry of Health & Family Welfare to further promote, preserve, protect & effectively utilize the knowledge available in the codified systems of medicine. A special task force is going to digitalise the knowledge to be called Traditional Medicine System knowledge database. Inventorisation & documentation of medicinal plants of India is also being looked after under newly constituted National Bioresource Development Board.

Indigenous Perspective and IPR

Given the source of knowledge in such cases as Azadirachta indica (Neem), the fairness of IPR law is being questioned by indigenous people. The draft UN declaration on Rights of Indigenous Peoples expresses the concerns, demands and aspirations of hundreds of indigenous peoples organisations around the world. Article 29 states that :

"Indigenous people are entitled to the recognition of the full ownership, control and protection of their cultural and intellectual property. They have the right to special measures to control, develop and protect their sciences, technologies and cultural manifestation including human and other genetic resources, seeds, medicines, knowledge of the properties of fauna & flaura, oral traditions, literature, designs & visual & performing arts"

Evidently, indigenous peoples interpret their cultural and intellectual property broadly, so that these encompass much more than knowledge, but also their cultural

heritage their biological resources & even their cells & DNA. Infact, indigenous people have their own regimes to regulate access to and control over knowledge & resources, that are often more sophisticated than those based on IPR or national sovereignty. According to the North American Indigenous Peoples organisation, the Four Directions Council (1996) :

"Indigenous peoples posses their own locally - specific systems of jurisprudence with respect to the classification of different types of knowledge, proper procedures for acquiring and sharing knowledge, and the rights and responsibilities which attach to possessing knowledge, all of which are embeded uniquely in each culture and its language."

For this reason, the Four Directions council argues that :

"Any attempt to device uniform guidelines for the recognition and protection of Indigenous Peoples knowledge runs the risk of collapsing this rich jurisprudential diversity into a single 'model' that will not fit the values, conceptions or laws of any indigenous society. A better approach..... would be for the international community to agree that traditional knowledge must be acquired and used in conformity with the customary laws of the peoples concerned."

This perspective has limited support in the CBD, which in article 10(C) requires contracting parties to :

"Protect and encourage customary use of biological resources in accordance with traditional cultural practices that are compatible with conservation or sustainable use requirements."

For indigenous peoples, then, protection of knowledge and resources, and continuation of customary law and practice, are central to maintenance of their cultural identity. Therefore, control over these is an aspect of human rights. This needs to be understood by all governments, companies and other institutions before they enter into negotiations for the use of biogenetic resources on territories of

indigenous peoples. These negotiations should certainly involve indigenous peoples. Often they do not.

Gathering and Documenting Ethnomedical Information

A vast source of information and knowledge on potentially useful plants is still intact with the tribal communities. A consolidated account for inventory of such plant needs to be prepared which represent sites of the climax vegetation and hold enormous species diversity that lies preserved on religious or ritualistic reasons. Some noteworthy work on these lines has been carried out by various workers in India to bring out directories, bibliographies, dictionaries, state-of-art reports and review work.

Methodology

a) Geographical coverage

Survey should cover the entire area. In order to cover the great diversity of the region, it could be divided into a number of areas according to climate, floristic, demographic, economic and traditional factors.

b) Participants

Emphasis needs to be placed on traditional healers and also on the sector of population using home - remedies. As such mothers and grandmothers participate in the survival of traditional medicine within the family unit. Individual surveys should be conducted by a team consisting of Pharmacist, nurse, taxonomist, ethnolinguist etc.

c) Questionnaire

This should be designed keeping in view the local conditions, a sample is shown in Annexure I. Open ended questions are asked. The respondent or informant is made free to tackle the pathology of his or her choice. The interviewer needs to attempt targeted questions, to obtain the most accurate information about the treatment used for a specific disease, parts of the plant (s), preparation of the medicine, dosages etc.

d) Collection of plants

Plants with medicinal uses are collected by the team during survey. These are pressed and correctly identified by the taxonomist. The voucher specimens are deposited in the Regional or National herbarium.

Indexing and Abstracting of Information

Global interest in ethnomedical knowledge has given boost to research efforts and thus literature in ethnopharmacology has been increasing at a fast rate. The information is vast and lies scattered in various books, periodicals, proceedings, conference papers, reports, research highlights etc. The exponential growth of ethnobotanical literature has posed some problems in effective dissemination of information. Hence, there is an urgent need for setting up an information system for collection, processing, storage, retrieval and dissemination of information on ethnomedical knowledge. Indexing and abstracting services are important links in the chain of communication between the originator of information and the ultimate users. Factors that have contributed to the launching of such services are :

a) Enormous growth of published literature
b) Increasing specialization in all branches of knowledge.
c) Diversity of publication
d) Wide scatter of published information.

The indexing techniques aid in the storage and retrieval of information, and abstracting provides access to the vast reservoir of information, discipline-wise. The INDEXING SERIALS present only a list of references to primary or other sources related to specific subjects. The references or entries are generally arranged by topics or by any classification system. The ABSTRACTING SERIALS provide a concise summary of significant information in the document. These abstracts are arranged by broad subject categories. Indexing services facilitate identification of published documents with all the bibliographic details, thus providing access to relevant literature. Abstracting services, while performing the basic access function, aid a user in deciding whether or not the user should seek a copy of the original document for study or consultation. Both of these services facilitate :

A) Retrospective searches through their annual or other periodic cumulations.

B) Selection of specific document for photocopies for study.

C) Selection of documents for obtaining translations from them, and

D) Preparation of subject bibliographies

Need and Scope of Indexing and Abstracting Services

A vast store of information and knowledge on potentially useful plants is still intact with the tribal communities. These plants are used for various purposes, such as food, medicine, cosmetics, dyes beverages etc., index of such plants should be prepared which will give an overall idea of the potential value. Although some indices on ethnobotanical terms, tribes and plants used by different tribes are available, there is still further scope for preparing indexes and abstracts on the following areas :

1. Index on different tribes
2. Detailed information on selected tribes
3. Index of journals, books and other literature
4. Index on regional contributions
5. Index on particular plant, genus, family
6. Indexing of Abstracts
7. Directory of contributors
8. Inventory of sacred groves and mythological associations
9. Inventory of multipurpose plants, such as articles of personal adornment, dyes, tools domestic use etc.

Need for Establishing Database on Ethnomedical Knowledge

A database consisting of index and abstracts on published papers on ethnobotany and related subjects may be created. This will facilitate the current and retrospective literature search services. During the last two decades abstracting and indexing services have been helping in dissemination of information. However, baffling

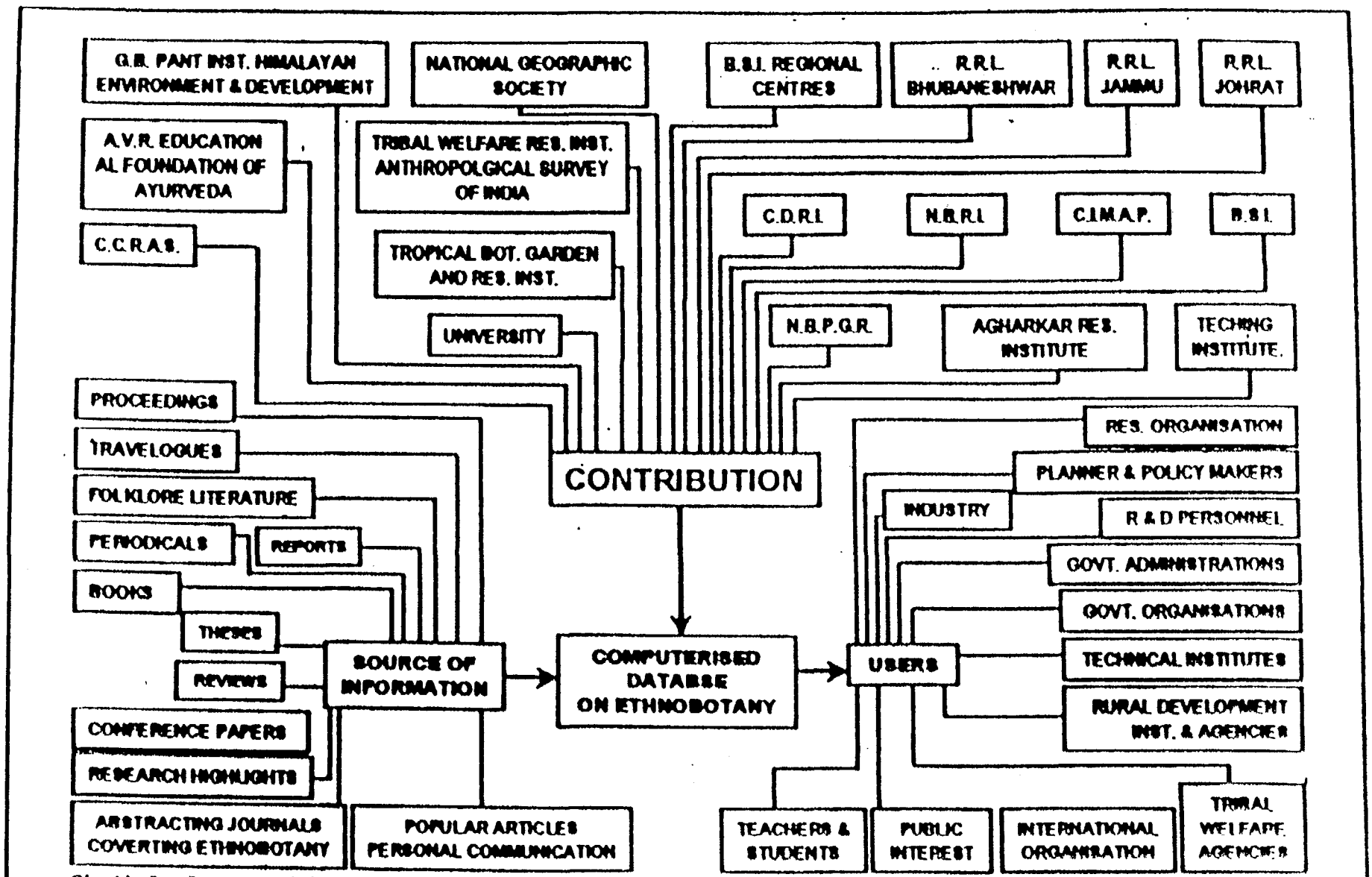

Chart Indian Sources Of Information, Contributors Of The Database On Ethnobotany And Its Users

complexity of information being generated has caused enormous strain on resources and capabilities of these information communicating systems.

The manual method of abstracting and indexing has become strenuous with the vast information available. Hence, a computerised indexing and abstracting on "Database on Ethnomedical Knowledge" may be created to provide information, and to build up storage, retrieval and dissemination capabilities. Interaction with various organisations is necessary to bring out all the information at one place. Chart I shows the sources of information, contributors to the "Database on Ethnomedical Knowledge" in India and its users. The database may be named '**ETHNO-MED NET**' and may store catalogue on books and literature published on ethnopharmacology, ethnobotany ethnofolklore in India. An user awareness programme may be conducted across the country to create awareness amongst ethnopharmacologists / ethnobotanists about the database and indexing and abstracting services as well as database on ethnopharmacology / ethnobotany will enable dissemination of retrospective / current; technical and techno-economic information.

Sources of Information on Medicinal Plants

a) Primary Publication

These are documents reporting current work or reviewing and analysing recent advances in knowledge. Documents in this category include journals reporting the results of original research, conference proceedings, annual and other reports published by various organisations, books, theses and patents. Approximately a quarter of the total volume of literature currently being generated on medicinal and related groups of plants appears in less than ten periodicals. Approximately 50% of the total volume is contained in some 50 titles. However, the remaining 50% of this literature is scattered across some 2,300 periodicals in a wide range of disciplines. Extra MED, the WHO - sponsored CD-ROM publication containing the text and the illustrations of papers from over 290 medical journals published in developing countries (India, Pakistan, China, Thailand, Philippines, Hong Kong, Korea, Singapore, Tanzania, Saudi Arabia etc.). Extra-MED provides better coverage of

literature in areas such as traditional medicines tropical medicines, AIDS, waterborne diseases etc.

b) Secondary Information Databases

Information on medicinal and other related groups of plants (which include herbs, spices and condiments, essential oils, plants containing compounds exhibiting insecticidal, molluscicidal, piscicidal, antifungal, antibacterial, antiviral and other biocidal activities) can be found in bibliographic databases dedicated exclusively to these groups of plants, as well as in botanical, biological, agricultural, chemical, medical, veterinary or multidisciplinary data bases with much wider subject coverage. The most important among these are listed below :

1. **MAPA** : Medicinal and Aromatic plants Abstract (MAPA) published by (NISCOM) National Institute of Science Communication, New Delhi, India., Contact : National Institute of Science Communication, Dr. K.S. Krishnan Road, New Delhi - 110012, India. Tel : (011) 574 - 6024 ; Fax : (011) 578 - 7062

2. **CAB ABSTRACTS** : Among multidisciplinary bibliographic data bases, CAB Abstract provides all - round coverage of world literature on medicinal and related medicinal plants covering botany, agronomy, biotechnology, phytochemistry, economics, medicines and veterinary science. Contact : Marketing Department, CAB International, Wallingford, Oxon OX 10 SDE, U.K. Tel : + 44 1491 832111; Fax + 44 1491 833508

3. **AGRIS** : The AGRIS (International Information System for Agricultural Sciences and Technology) data base managed by the Library and Documentation systems Division of FAO, ROME, Contact : Dissemination Management Branch,1 via delle therme di Caracalla 00100, Rome, Italy. Tel : (396) 5705 4993 ; Fax : (396) 57054049.

4. **AGRICOLA** : Bibliographic database compiled and maintained by the US National Agriculture Library, Beltsville, Maryland. Contact : National Agriculture Library, 10301 Baltimore, Boulevard, Beltsville, MD 20705 - 2351, USA.

5. **PASCAL** : Compiled by the Institute de l information scientifique et technique (INIST) of French centre. National de la Researche Scientifique. Contact : INIST DIFFUSION, 2 Allee du Parc de Brabois 54514 Vandceuvre Cedex, France. Tel : + 33 83 504664; Fax : + 33 83 50 46 66.

6. **BIOSIS PREVIEWS** : Probably the world's largest bibliographic information data base on biological subjects including medicine compiled by BIOSIS of Philadelphia, USA, Contact : BIOSIS, 2100 Arch street, Philadelphia, PA 19103-1399. Tel : + 1 215 - 587 - 4847; Fax : + 1 215 587 - 2016.

7. **CHEMICAL ABSTRACTS** : For phytochemical information on medical plants and patent related literature, Chemical Abstract is an indispensable resource. Compiled by CAS (a division of American Chemical Society) in Columbus, OHIO, Contact : Chemical Abstract service 2540 Olentangy River Road, P.O.Box 3012, Columbus, OHIO, USA, Tel : + 1 614 447 3600; Fax : +1 614 4473713

8. **MEDLINE** : A limited amount of information relating to medicinal plants (from mainstream medical literature) can be found in this most widely known database of medicine compiled by National Library of Medicine, USA. Contact : The National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 208 94 USA, Tel : 800 - 272 - 4787; or 301 - 496 - 6308

c) Tertiary Information Databases

Unlike the secondary information data bases listed above, tertiary electronic publications containing information on medicinal related group of plants vary widely in their design, structure and contents. A number of them produced for different purposes by various organisations, are listed below. While some of them can be accessed online, others are only available on CD - ROM or other offline electronic media.

1. **AHEAD CD-ROM** The Asian Health, environmental and Allied Databases (AHEAD) CD- ROM series consists of three disks containing various databases contributed by the participating organisations based in India, Thailand, Philippines, Malaysia, Singapore and Bangladesh. This has been sponsored by the International Development Research Centre of Canada and co-ordinated by NISCOM, New Delhi, India.

Disk 1 entitled "Environment Asia" contains full text and bibliographic databases related to water management recycling wastewater, hygiene education of community participation.

DISK 2 entitled "Wealth Asia", contains the entire medicinal and aromatic plants bibliogr database mentioned above and a full - text data base of Indian plant, animal and mineral sou based on the well - known "Wealth of India" encyclopedic book series.

Disk 3 is called "Health Asia " and contains a bibliographic database on tropical (mosquito - transmitted) diseases and occupational safety and health, a full-text database on waterborne (diarrhoeal) diseases, and a natural toxin database providing text and pictorial information on poisonous plants and animals. Contact : The Executive Director, AHEAD, NISCOM. Dr. K.S. Krishnan Marg, New Delhi 110012, India. Telephone : (011) 572 - 8385 ; Fax : (011) 5787062

2. **APINMAP** : The Asia Pacific Information Network on Medicinal and Aromatic Plants (APINMAP) is a UNESCO- sponsored voluntary network of organisations in 14 Asian and Pacific region countries (Australia, People's Republic of China, India, Indonesia, Republic of Korea, Malaysia, Nepal, Pakistan, Papua New Guinea, the Philippines, Sri Lanka, Thailand, Turkey and Vietnam) with a Secretariat based in the Philippines. Its objective is to promote exchange of information relating to medicinal and aromatic plants between its member organisations. Databases and other resources held by each organisation are shared with others. APINMAP resources include an Integrated APINMAP database containing bibliographic and factual information on medicinal plants, lists of research projects, institutions and

personnel. Contact : Secretary General, APINMAP Philippine Council for Health Research and Development, Department of Science and Technology, Bicutan, Tagig, Metro Manila, Philippines. Telephone: Manila 837-29-42. Internet homepage : http://www.pchrd.dost.gov.ph/apinmap/

3. **BACIS** : Boelens Aroma Chemical Information service (BACIS) offers a set of five databases (which can be installed on a desktop PC) mainly aimed at users in the perfumery and flavouring industries. These contain information on volatile compounds in foods, analytical chemical data compiled from published literature on essential oils and other natural compounds, and trade related data. Contact : BACIS, Groen van Prinstererlaan 21, 1272 GB Huizen, The Netherlands, Telephone and Fax : +31 2152 53558.

4. **BIOLOGICALLY ACTIVE PHYTOCHEMICALS AND THEIR ACTIVITIES; and PHYTOCHEMICAL CONSTITUENTS OF GRAS HERBS AND OTHER ECONOMIC PLANTS** A set of two databases compiled by Dr. James Duke of ARS/USDA. The first of these contains information on some 3000 biologically active (medicinal, antimicrobial, pesticidal and allelopathic) phytochemicals, their reported activities and inhibitory concentrations or doses. The second database lists the chemical constituents of approximately 1000 plant species. These include most of the GRAS, (generally recognised as safe) plants, many medicinally important foods GRAF (generally recognised as foods) and about 500 strictly medicinal GRAP (generally recognised as poisonous or medicinal plants).

These databases are published in book form with accompanying diskettes, by CRC Press, Inc. Information contained in these databases can be searched online on the **Phytochemical Database** (which also has input form other interconnected databases) on the Internet at the following URL: http/probe.nalusda.gov.8300/cgi-bin/query?dbname=phytochemdb, Contact : CRC Press Inc., 2000 Corporate Blvd., N.W., Boca Raton, FL 33431, USA.

5. **BRAZILIAN MEDICINAL PLANTS DATABASE** : This is a database being developed by the Medicinal Plants Laboratory of the Escola Superior de Agricultura "Luiz de Queiroz", Sao Paulo University, and CIAGRI, the computing centre of the university. It currently contains the common name in Portuguese, Latin name and Synonyms, family, biological activity, and therapeutic uses of over a thousand species. Contact : ESA "Luiz de Queiroz", Piracicaba, Dao Paulo, Brazil. Internet URL: http://www.ciagri.usp.br/planmedi/planger.htm

6. **CHINA ACADEMY OF TRADITIONAL CHINESE MEDICINE DATABASES** : The Institute of Information of the China Academy of Traditional Chinese Medicine has put together several bibliographic and factual information databases. The largest among these is the **Traditional Chinese Medical Literature Analysis and Retrieval System** (TCMLARS), which contains about 200,000 bibliographic records of literature on traditional Chinese medicine and Western medicine, published in over 500 Chinese and foreign biomedical journals since 1984. TCMLARS consists of its three constituent databases, the largest among these being the **Traditional Chinese Medical Literature** (TCM) database. The other two are the **Acupuncture Literature Analysis and Retrieval System** (ACULARS), and the **Chinese Materia Medica** (CMM) database which contains chemical, pharmacological and horticultural information on medicinal plants. TCMLARS has both English and Chinese versions.

Traditional Chinese Patent Drug and Health Products database (which contains about 2000 entries relating to the production and marketing of traditional Chinese patent drugs and health products), and **Aids Information** database and an **Overseas TCM Academic Organisations and Scholars** database. Contact : Retrieval Section, Institute of Information on Traditional Chinese Medicine, China Academy of Traditional Chinese Medicine, 18 Beixincang, Dongzhimen Nei, Beijing, 100700, P.R. China. Telephone: (10) 403 2167; Fax: (10) 403 2167; E-mail: wulc@sun.ihep.ac.cn

7. **CHINESE UNIVERSITY OF HONG KONG DATABASES** : The Chinese Medicinal Material Research Centre (CMMRC) of the Chinese University of Hong Kong has compiled a database of TCM which contains botanical, chemical, pharmacological and clinical information selected from Chinese medical treatises and translated into English. This database is updated by abstracting papers form over 180 medical and other scientific journals in Chinese. CMMRC is also developing a database on the safety of Chinese food and medicines. The first product is CHIMERA, a bibliographic database on reported cases of adverse reactions to Chinese foods and medicines. Colour images of the suspected materials are also included in this database. Contact : Chinese Medicinal Material Research Centre, The Chinese University of Hong Kong, Hong Kong. E-mail: cmmrc@cuhk.edu.hk

8. **DICTIONARY OF NATURAL PRODUCTS** : DNP on CD-ROM published by Chapman and Hall contains chemical, physical, bibliographic and structural data on over 113,000 natural products, organised into over 36,000 entries. Pharmacologically active compounds, food ingredients and many compounds of biochemical significance are well covered. Contact : Chapman and Hall, 2-6 Boundary Row, London SEI 8HN, UK. Telephone: +44 171 865 0066; Fax: +44 171 522 9621. Internet homepage : http://www.thomson.com;8866/chaphall/default.html

9. **DIRECTORY OF SPECIALISTS IN HERBS, SPICES AND MEDICINAL PLANTS** This is a database containing names, addresses, professional expertise and interests, and contact details of specialists in this field, compiled by Professor Lyle Craker, University of Massachusetts, Amherst. It is available in printed form. Contact : Professor L E Craker, Department of Plant and Soil Sciences, University of Massachusetts, Stockbridge, amherst,MA 01003, USA. Telephone: +1 413 545 237; Fax: +1 413 545 1242

10. **LANGER'S DROGENANALYSE** : Published by Deutscher Apotheker Verlag, Stuttgart, Germany, is a plant identification aid database on floppy disk. It contains name, synonyms and illustrations of medicinal and poisonous plants (2,400 entries

in total) Contact: Deutscher Apotheker Verlag, Postfach 10 1061, 70009 Stuttgart, Germany Telephone: (0711) 25 82 347 oder 257 Fax: (0711) 25 82 290

11. **ETHMED** is a database currently being compiled at the Yakko Kaiseki Centre (Analytical Research Centre for Ethnomedicines) of the Institute for Wakan- Yaku (Traditional Sino Japanese Medicines) affiliated to the Faculty of Pharmaceutical Sciences of Toyama University, Japan. Data on morphology, anatomy, active principles, biological activity and uses of medicinal plants are being recorded. The project is linked to the cataloguing and indexing of the crude drug samples held at the Museum of Materia Medica of the Institute, which is the largest museum of its kind in the world. The museum holds over 20,000 crude drug samples, more than 75,000 herbarium specimens, and other pharmaceutical preparations covering virtually every system of traditional and folk medicine practised around the world. Contact : Analytical Research Centre for Ethnomedicines, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. Fax: 0764-34-5055; Internet homepage:http://www.toyama-mpu.ac.jp

12. **FLAVOUR AND FRAGRANCE MATERIALS** is a chemical entry database published by Allured Publishing Corporation. The database contains 2,500 records with CAS registry numbers, structure, molecular formulae and physical constants for flavour and fragance materials. Contact : Allured Publishing Corporation, 362 South Schmale Road, Carol Stream, IL 60188-2787,USA. Telephone : (708) 653 2155; Fax: (708) 653 2192; Internet URL: http://www.barnaby.com/cosmetic.html

13. **FLORIN MEDICINAL PLANTS** : The database is compiled by Professor Boris Golovkin of the Moscow Botanic Gardens. It is one of the several taxonomic and economic botany databases published by Forin, Inc. of Moscow.
FLORIN MEDICINAL PLANTS now contains information on over 5,000 taxa of vascular plants from more than 200 families and is expected to grow to twice its current size in the near future. Taxanomic data, plant parts used, bioactive substances they contain and their therapeutic activity or toxicity are among the

interactively searchable fields. Classified lists of diseases and drugs are also included. Contact : DataX/FLORIN,Inc., Moscow, Russia. Telephone: (095) 158-9520; Fax: (095)158-5700; Internet homepage:http://www.florin.ru

14. **FLOTURK** : FLOTURK (FLOra of TURKey) is a database compiled and maintained by the Anadolu University Medicinal Plant and Drug Research Centre (TBAM). It contains botanical, phytochemical, chemotaxonomic and pharmacological activity-related data as well as information on production and commercial potential of Turkish flora. Contact : Anadolu University Medicinal Plant and Drug Research Centre, Eskisehir 26470, Turkey. Telephone +90 222 335 2952; Fax: +90 222 335 0127; Internet hompage : http:// www.anadolu.edu.tr/anadolu/ tbam/index.html

15. **GREEN MEDICINE** : is a Chinese Herbal Medicine database containing information on 390 biomedical syndromes, 257 basic formulas, 490 individual herbs and 600 variations, Contact : The Journal of Chinese Medicine, 22 Cromwell Road, Hove, Sussex, England. Fax : +44 1273 748588

16. **HOPKINS TECHNOLOGY CD-ROMs** : The series of multimedia CD-ROMs published by Hopkins Technology of Hopkins, Minnesota, include (1) the HERBALIST and (2) the Traditional Chinese Medicine and Pharmacology databases.

The HERBALIST by David Hoffman is an encyclopaedia of western herbal medicine and gives botanical information on the plants used as well as medical and pharmacological information relating to their use. The *Materia Medica* consists of data sheets on some 70 plant species illustrated with colour pictures and containing the following information: Latin and common names, method of collection, parts used, constituents, pharmacological activity, preparations and dosage. The Traditional Chinese Medicine & Pharmacology CD-ROM describes the basic philosophical elements, and diagnostic and therapeutic principles of TCM. The *Materia Medica* gives information on the use of 322 medicinal herbs with colour illustrations. Commonly used formulas are given with their functions and applications. Contact : Hopkins Technology, LLC, 421 Hazel Lane, Hopkins, MN

55343-7116 USA. Telephone (612) 931 9376. Fax: (612) 931 9377. Internet homepage: http://www.ncptechno.com/

17. **IMMEDPLAN** : IMMEDPLAN (Indian Medicinal Plants National Network of distributed data bases) is an initiative of a network of several Indian organisations with expertise on different aspects of medicinal plants to build a multidisciplinary (botanical, horticultural, pharmacological and other) information pool by sharing their resources. The network secretariat is at FRLHT, Bangalore. Contact : Foundation for Revitalisation of Local Health Traditions, 50 MSH Layout, Anandnagar, Bangalore 560 024, India. Telephone: +91 80 333 6909; Fax: +91 80 333 4167; Internet homepage: http://ece.iisc.ernet.in/ernet-members/frlht.html

18. **MAPI** : MAPI (Major Aromatic Plants of India) is a database compiled by the Central Institute of Medicinal and Aromatic Plants, Lucknow. It contains very detailed botanical, agronomic, phytochemical and bibliographic information on 45 major aromatic plants of India. The database has a very elaborate structure with a total of 86 unique data entry fields for each record. Contact : Central Institute of Medicinal and Aromatic Plants, P B No. 1, RSM Nagar, Lucknow 226016, India. Telephone : +91 522 71170; Fax: +91 522 73654; Internet homepage: http://www.sunsite.sut.ac.jp/asia/india/jitnet/india/csir/cimap.html

19. **MEDICINAL PLANTS OF MALTA** : An electronic inventory of 300 medicinal and aromatic plants of Malta, compiled by the University of Malta, containing text and images. Contact : Royal University of Malta, Msida, Malta.

20. **MEDICINAL PLANTS OF PAPUA NEW GUINEA** : Developed by the Wau Ecology Herbarium, this database contains botanical, phytochemical and ethnopharmacological information on plants native to PNG. Contact : Wau Ecology Institute, P O Box 77, Wau Papua New Guinea.

21. **NAPRALERT, MEDFLOR and DEREP** : These are three inter-related databases developed by the Department of Medicinal Chemistry and

Pharmacognosy of the College of Pharmacy, University of Illinois at Chicago, USA. The NAPRALERT (NAtural PRoducts ALERT) database contains bibliographic and factual data on natural products of plant, microbial and animal origin. It is compiled from ethnomedical source literature scanned from some 125,000 journal articles, books, abstracts and patents covering the period from 1975 to date. Some information derived from older literature dating back to 1650 is also included. The database as an elaborate field structure and provides extensive information on the chemistry, pharmacology, biological activity, taxonomic and geographical distribution and ethno-medical uses of some 110,000 natural products and 120,000 organisms. Formation from approximately 600 new articles scanned are added every month.

MEDFLOR (MEDicinal FLORa) is an ethnobiological database being developed in collaboration with the Organization of American States. It aims to compile botanical information and ethnomedical uses of plants from literature scanned locally at data entry sites located in Costa Rica, Panama, Trinidad and Tobago, Hungary and India. DEREP (DEREPlication) is a recently initiated database exclusively containing data on the physical constants of natural products. Online search subscriptions to NAPRALERT are available through STN International and other online vendors. A CD-ROM version of NAPRALERT has been announced for 1997 by Chapman and Hall. Access to MEDFLOR and DEREP is restricted to collaborating organisations. Contact : Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60680. Telephone: +1 312-996-5381; Fax: +1 312-996-7107. Internet homepage: http://pcog8.pmmp.uic.edu/mcp/MCP.html

22. **NATTS**: NATTS is the Natural Products database developed by the Central Drug Research Institute, Lucknow. It contains factual information on medicinal plants. Detailed data on botanical characters, collection site details, pharmacological screening results, chemical structures of active constituents, uses in folk medicine and in established traditional medicine, information from traditional health systems' literature and from modern scientific literature are recorded in the six sub-files constituting the database. Contact : Documentation and Library

Services Division, Central Drug Research Institute, Chattar Manzil Place, Lucknow 226 001, India. Telephone: Lucknow 234219; Telegram: CENDRUG, LUCKNOW; Telex : 0535-286/0535-344 CDRI IN; Email: 23. PLANTES MEDICINALES
This multimedia CD-ROM database developed as an educational tool by Professor Michel Paris of the Department of Pharmacognosy, Faculty of Pharmacy, Chatenay-Malabry,France is sponsored by the French Ministry of Education and published by Algo Vision, Paris. It contains botanical and phytotherapeutic information on some 175 species and is well illustrated with over 500 high quality colour photographs. Descriptions of pathological conditions are accompanied by monographs on the main species used in the treatment and additional lists of other relevant plants. The French version is expected to become available by June 1997. An English version is also being planned. Contact : Societe ALGO VISION, 27 rue du Chateau d'eau, 75010 Paris, France. Telephone: +33 1 44 84 03 03; Fax: +33 1 44 84 06 13. E-mail: algovisi@pratique.fr

24. **POISONOUS PLANTS IN BRITAIN AND IRELAND** : This is a CD-ROM database developed jointly by the Poisons Unit of Guy's & St Thomas' Hospital Trust, London and the Royal Botanic Gardens, Kew. It is an interactive database designed for identifying common poisonous plants using easily recognisable morphological features such as size, shape and colour of different plant parts for characterising the species concerned. The database contains textual and pictorial information on over 200 plant groups covering approximately 2,000 species and cultivars.

Two versions have been published, a medical version for the benefit of medical professionals dealing with suspected plant poisoning cases, especially in hospital Accidents & Emergency Departments for identifying plants ingested accidentally by children, and a more popular version aimed at the general public. Contact : The Stationery Office, Electronic Publishing Sales, 51 Nine Elms Lane, London SW8 5DR, UK. Telephone: + 44 171 873 8236; Fax: + 44 171 873 8203. URL: http://194.128.65.2/publicat/titles/plants/plants.htm

25. **PROSEA** : PROSEA (PLANT RESOURCES OF SOUTH-EAST ASIA) is a foundation with an international charter and consists of a network of participating organisations based in Indonesia, Malaysia, Papua New Guinea, Philippines, Thailand, Vietnam and the Netherlands. The network has a secretariat is at Bogor, Indonesia, and a publishing office at Wageningen, Netherlands.

The main objective of PROSEA is to collect and disseminate information on the plant resources of South-East Asia for education, extension, research and industry. Its main activities therefore involve developing electronic databases and publishing books, CD-ROMs, bibliographies etc.

The PROSEA database already contains a wealth of information on some 6,000 useful plants of South-East Asia. A series of scholarly handbooks, CD-ROMs and bibliographies on several commodity groups, and other products derived from the database have already been published. Future publications will include volumes on spices and condiments, medicinal and poisonous plants, essential oil plants, stimulants, and plants producing exudates among others. Contact : PROSEA Network Office, c/o Research and Development Centre for Biology (RDCB-LIPI), Jalan Ir. H. Juanda 22, P.O.Box 234, Bogor 16122, Indonesia. Telephone:+62 251 322859; Fax: +62 251 370934

or

PROSEA Publication Office, Wageningen Agricultural University (WAU), Haarweg 333, P.O.Box 341, 6700 AH Wageningen, The Netherlands. Telephone: +31 317 484587; Telex: 45917 BURLU; Fax: + 31 317 482206; Internet homepage: http://www.bib.wau.nl/prosea/home.html

26. **SEPASAL** : The SEPASAL (SURVEY OF ECONOMIC PLANTS FOR ARID AND SEMI-ARID LANDS) database developed by the Centre for Economic Botany, Royal Botanic Gardens, Kew, is a major source of information on the flora of arid and semi-arid regions and a valuable resource for people involved in biodiversity conservation, germplasm collection and storage, and environmental management.

It contains information gathered from various sources on some 6,000 useful dryland species. The data include detailed botanical descriptions, geographic distribution, conversation status, soil and climatic preferences, and uses of different plant parts

following an international standard classification. The range of information and the amount of data vary between species. Contact : Centre for Economic Botany, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK. Telephone: +44 181 332 5719; Fax: +44 181 332 5278; Internet homepage: http://www.rbgkew.org.uk/ceb/ceb.html

27. **TRADIMED** : TRADIMED is a TCM database developed by the Natural Products Research Institute of the Seoul National University. The database contains pharmacological data (efficacy, dosages, adverse effects) for traditional Korean and Chinese drugs, chemical data (composition and structural formulae) for natural products from botanical, microbial and marine organisms, can colour images of medicinal plants. An English version of the database is available on CD-ROM. Contact: Natural Products Research Institute, Seoul National University, 28 Yeongun-Dong, Jongro-Ku, Seoul 110-460, Republic of Korea. Telephone: +82 2 740 8901; Fax: +82 2 742 9951;URL:http://yes.snu.ac.kr/TRadiMedENG.htm

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Mitra R. (1996) Role of Indexing & Abstracting services in Dissemination of Ethnobotanical Information. In Ethnobiology in Human Welfare (Ed. S. K. Jain), Deep Publications, New Delhi, India pp. 441 - 445

Bhat K.K.S. (1999). Medicinal Plant Information databases. In Medicinal plants for conversation & Health Care - pp. ***

Handa S. S. (1998). Protecting Intellectual Property Rights for Indigenous systems of medicine. Lecture delivered at a special symposium organised by The Department of Indian Systems of Medicine, Ministry of Health & Family Welfare Government of India.

Swaminathan M.S. (1994). Rights, Rewards and Recognition. Our Planet 6 (4), 11-13.

SAMPLE-SURVEY-QUESTIONNAIRE

PART I : SOCIO-ECONOMIC CONDITION

SURNAME.....FIRST NAME.....

AGE..... TOWN.....

SOCIO-PROFESSIONAL STATUS.....

PART II : **HEALTH PROBLEMS**

1) NAME OF HEALTH PROBLEM.....
2) TRADITIONAL DESCRIPTION OF THE DISEASE AND ASSOCIATED PRECAUTIONARY MEASURES.....
3) THE LAST TIME YOU OR SOMEBODY IN YOUR FAMILY, HAD THIS HEALTH PROBLEM, WHAT DID YOU DO ?.....

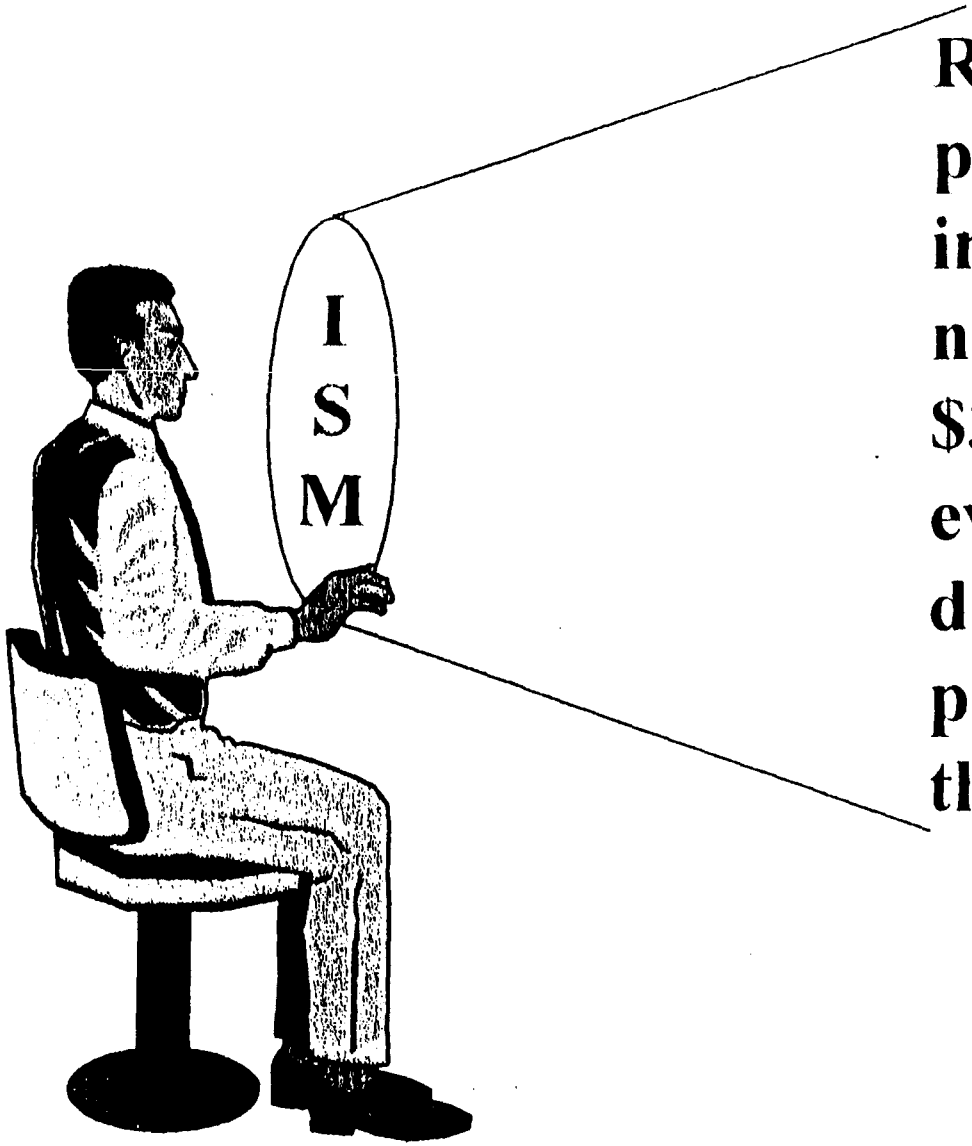
a) DOCTOR b) WITCHCRAFT HEALER c) MEDICINAL PLANT

[ANSWER a) AND b) LEAD TO THE END OF THE SURVEY FOR THIS HEALTH PROBLEM

SAMPLE-SURVEY-QUESTIONNAIRE (Contd.)

PART II : **HEALTH PROBLEMS**

4) WHAT IS (OR ARE) THE PLANTS YOU USE ? _____
[IF SEVERAL PLANTS ARE USED TOGETHER, USE ONE FORM PER PLANT, BUT NOTE DOWN ON EACH FORM ALL PLANTS]
5) WHERE DID YOU GET THE PLANT_____
[I CULTIVATED AROUND THE HOUSE II = WILD BUT AROUND THE HOUSE
III = IN THE NEIGHBOURING COUNTRYSIDE IV = WOOD FOREST
V = FROM THE MARKET VI = ANY OTHER SOURCE]
6) GATHERING SEASON AND/OR HOUR
7) PART (S) OF PLANTS USED TO PREPARE THE REMEDY_____
[FL = WHOLE PLANT; ST = STEM; BK = BARK; RT = ROOT;
WD = WOOD; SD = SEED; LF = LEAF; FR = FRUIT;
BD = BUD; FL = FLOWER; LA = LATEX OR RESIN,
AP = AERIAL PARTS; NT = NUT]
8) QUANTITIES OF PLANT USED_____
9) ADMINISTRATION FORMS_____
[INF = INFUSION; DE = DECOCTION; MA = RAW
MA = AQUEOUS STEEPING; TR = ALCOHOLIC STEEPING
JU = JUICE; PP = POUNDED, CRUSHED OR GROUND;
PO = POWDER ; HU = OIL; SY = SYRUP; BL = BROTH]
10) ADMINISTRATION MODE_____
[VO = ORAL; AP = APPLYING, POULTICE; FR = RUBBING OR MASSAGE;
BA = BATH; IN = INHALATION; IN = INSTILLATION]
11) QUANTITY OR DOSAGE OF REMEDY TAKEN EACH TIME_____
12) HOW MANY TIMES A DAY_____
13) FOR HOW MANY DAYS_____
14) CAN THIS REMEDY BE DANGEROUS ?_____
ARE THERE ANY CONTRAINDICATIONS ?
[I = YES; II = NO; III = DOES NOT KNOW]



R.A.F.I. Estimates that the pharmaceutical industry of the Developed nations already owes $32billion (Rs.1,12,000 cr) every year to the developing nations on plant based drugs used by them.

INDIA'S PROBLEMS OF PATENTING ISM DRUGS

- **COUNTRY WILL FACE PROBLEMS WHILE PATENTING PLANT-BASED PRODUCTS AND PROCESSES AS PHARMCEUTICAL COs INCREASINGLY SCREEN INDIA'S MEDICINAL PLANTS FOR NEW DRUGS.**

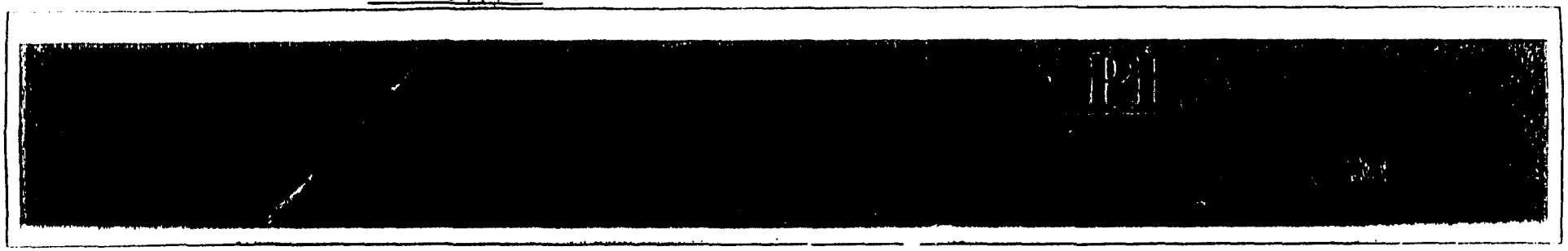
- **NO COUNTRY WILL GRANT PATENT ON PRODUCTS WHICH HAVE BEEN USED ANY WHERE IN THE WORLD**

- **NO COMPANY CAN OBTAIN A PATENT ON ANY FORMULATION USED IN AYURVEDA OR ANY OTHER SYSTEMS OF MEDICINE IN INDIA AS A MERE REFERENCE IN AN ANCIENT TEXT IS ENOUGH CAUSE FOR REJECTION**

Contd....

BIOPIRACY OF INDIAN PLANTS

UNABATED



U.S. TOPS THE LIST WITH MAXIMUM NUMBER OF PATENTS FOR INDIAN PLANTS FOLLOWED BY JAPAN, CANADA, FRANCE, GERMANY AND U.K. PATENTS ALREADY OBTAINED BY THESE COUNTRIES ON 100 PLANTS SUCH AS *Cassia fistula*, *Momordica chirantia*, *Aloe barbedensis*, *Sapindus mukorossi*, *Punica granatum*, *Boswellia serrata*, *Euphorbia hirta*, *Impatiens balsamina*, *Solanum nigrum*, *Terminalia arjuna*, *Terminalia chebula*, *Jatropha curcas*, *Tinospora cardifolia*, *Withania somnifera*, *Tribulus terrestris*, *Phyllanthus amarus*

PATENTING TREND MED. PLANT PRODUCTS

- OUT OF 750 PATENTS (1980-1992) 566 ACCOUNT FOR MEDICINAL AND 184 FOR AROMATIC PLANTS
- THRUST ACTIVITY AREAS INCLUDE ANTICANCER, ANTIINFLAMMATORY, CARDIAC ACTIVITY, CNS, ANTIMICROBIAL, HYPOGLYCAEMIC AND ANALGESIC
- COSMETICS - HAIR DYE, PREVENTION OF HAIR LOSS, HAIR SHAMPOO, SKIN CREAMS, EYE LOTIONS
- JAPAN HAS MAJOR SHARE OF PATENTS
- APPLE, MELON, WATERMELON, POTATO STARCH, CABBAGE, CARROT, SWEET POTATO HAVE BEEN PATENTED FOR VARIOUS PHARMACOLOGICAL ACTIVITIES

HANDA

PATENTS ON INDIAN INDIGENOUS MEDICINAL PLANTS:

Scientific & Common Name	Uses	Patent No. and Use
Boswellia serrata ***Salai (Hindi)*** **Indian Olibanum Tree (Eng.)**	**Astringent, helpful in the treatment of skin diseases, piles and ulceration.**	**(5494668) Method for treating muskuloskeleton & a novel composition thereof.**
Curcuma longa ***Haldi* (Hindi)** **Tumeric (Eng.)**	**Wound Healing**	**(5401504) Four patents have been filed, Used in the preparation of topical wound healer. Also used as an anti-inflammatory agent, food additive and in cosmetics.**
Phyllanthus emblica ***Amla* (Hindi)** **Indian Gooseberry(Eng.)**	**Relives fatigue, vomitting, latufence, constipation, & diarrhoea, allevias, burning, anaemia, jaundice**	**(55299778) Ayurvedic composition for the prophylaxis & treatment of AIDS, flu,TB & other immunodeficiencies & the process for preparaing the same.**
Azaadirachta indica ***Neem* (Hindi)** **Margosa Tree (Eng.)**	**Medicine, prophylactic, biopessticide, biofertiliser, biofungicide, nitrogen fixer for soil. Same properties as for Melia azadirachta.**	**(5405612) (4515785) (4537774) (4556562) (4902713) (4943434) (4946681) (4960791) (5001146) (5001149) (5009886) (5047242) (5110591) (512449) (436257 B1) There are 65 patents filed for this plant. Cures jaundice & hepatitis A,B,C,D & infective hepatitis. It has numerous properties like pesticidal,antiviral & several other prophylatic properties**
Phyllanthus niruri ***Jar Amla* (Hindi)**	**Beneficial for hepatitis, jaundice, liver disorders, urinary diseases, burning sensations, antiseptic, genital diseases, ulcers, dysentery & colic.**	**Patent still in process. Application for curing jaundice & viral hepatitis B & infective hepatitis.**

- **PROBLEMS GET FURTHER COMPLICATED e.g. B.S. BLUMBERG NOBEL PRIZE WINNER US VIROLOGIST HAS OBTAINED A PATENT FOR PHYLLANTUS AMARUS, A PLANT USED FOR CENTURIES IN SOUTH INDIA FOR TREATMENT OF JAUNCIDE. THE PATENT NOVELTY GIVEN BY BLUMBERG IS FOR VIRAL HEPATITIS WHICH IS DIFFERENT FROM JAUNDICE MENTIONED IN AYURVEDA - SO INNOVATION SATISFY THE CRITERION OF NOVELTY**

- **SIMILAR PATENTS WILL CONTINUE TO BE AWARDED ON ISM DRUGS SINCE THER IS NO SHORTAGE OF UNTAPPED WEALTH**

PATENTING RECIPES FROM NATURE'S KITCHEN

1. Have I purified compound from nature ?
2. Do I have an unpredictable use for a known natural component ?
3. Have I made an analogue to natural product ?
4. Have I isolated biologically pure culture or cell line ?
5. Have I finger printed something new or unexpected ?
6. Have I made a new combination ?
7. Have I created a new method of preparation ?
8. Have I created a new method of use for a known or even previously patented invention ?
9. Have I created a new a recombinant product that differs from the known naturally derived product ?
10. Have I created a new plant or animal ?

HANDA

INDIGENOUS PERSPECTIVES

ISSUES

- THE DRAFT UN DECLARATION ON RIGHTS OF INDIGENOUS PEOPLES EXPRESSES THE CONCERN DEMANDS AND ASPIRATIONS OF HUNDREDS OF PEOPLES ORGANISATIONS AROUND THE WORLD. ARTICLE 29 STATES THAT :

- INDIGENOUS PEOPLES ARE ENTITLED TO THE RECOGNITION OF THE FULL OWNERSHIP, CONTROL AND PROTECTION OF THEIR CULTURAL AND INTELLECTUAL PROPERTY.

- THEY HAVE THE RIGHT TO SPECIAL MEASURES TO CONTROL, DEVELOP AND PROTECT THEIR SCIENCES, TECHNOLOGIES AND CULTURAL MANIFESTATIONS, INCLUDING HUMAN AND OTHER GENETIC RESOURCES, SEEDS, MEDICINE, KNOWLEDGE OF THE PROPERTIES OF FAUNA AND FLORA, ORAL TRADITIONS, LITERATURES, DESIGNS AND VISUAL AND PERFORMING ARTS.

ACCORDING TO THE NORTH AMERICAN INDIGENOUS PEOPLES' ORGANISATION THE FOUR DIRECTIONS COUNCIL (1996)

"INDIGENOUS PEOPLES POSSES THEIR OWN LOCALLY-SPECIFIC SYSTEMS OF JURISPRUDENCE WITH RESPECT TO THE CLASSIFICATION OF DIFFERENT TYPES OF KNOWLEDGE, PROPER PROCEDURES FOR ACQUIRING AND SHARING KNOWLEDGE, AND THE RIGHTS AND RESPONSIBILITIES WHICH ATTACH TO POSSESING KNOWLEDGE ALL OF WHICH ARE EMBEDED UNIQUELY IN EACH CULTURE AND ITS LANGUAGE."

FOR THIS REASON, THE FOUR DIRECTIONS COUNCIL ARGUES THAT :

" ANY ATTEMPT TO DEVISE UNIFORM GUIDELINES FOR THE RECOGNITION AND PROTECTION OF INDIGENOUS PEOPLES' KNOWLEDGE RUNS THE RISK OF COLLAPSING THIS RICH JURISPRUDENTIAL DIVERSITY INTO A SINGLE 'MODEL' THAT WILL NOT FIT THE VALUES CONCEPTIONS OR LAWS OF ANY INDIGENOUS SOCIETY. A BETTER APPROACH..... WOULD BE FOR THE INTERNATIONAL COMMUNITY TO AGREE THAT THE TRADITIONAL COMMUNITY TO AGREE THAT THE TRADITIONAL KNOWLEDGE MUST BE ACQUIRED AND USED IN CONFORMITY WITH THE CUSTOMARY LAWS OF THE PEOPLES CONCERNED."

THE KARI-OCA DECLARATION

[ADOPTED BY THE KARI-OCA CONFERENCE OF INDIGENOUS PEOPLES AT UNCED IN 1992]

- INDIGENOUS WISDOM MUST BE RECOGNISED AND ENCOURAGED.
- THE TRADITIONAL KNOWLEDGE OF HERBS AND PLANTS MUST BE PROTECTED AND PASSED ON TO FUTURE GENERATIONS.
- TRADITIONS CANNOT BE SEPARATED FROM LAND, TERRITORY OR SCIENCE
- INDIGENOUS COMMUNITIES REQUIRE THAT THEIR RIGHT TO INTELLECTUAL AND CULTURAL PROPERTIES BE GUARANTEED AND MUST INCLUDE THE RIGHT OVER GENETIC RESOURCES, GENE BANKS, BIOTECHNOLOGY AND KNOWLEDGE OF BIO-DIVERSITY PROGRAMMES.

PROTECTING INDIGENOUS DRUGS UNDER IPR
MAJOR RECOMMENDATIONS

- INVENTORY OF MEDICINAL PLANTS INDIGENOUS TO INDIA AND USED IN ISM
- PRESERVATION OF GERMPLASM OF ALL PLANTS USED IN ISM
- ADOPT MODERN METHODS FOR PREPARATION, QUALITY CONTROL WITH PROVEN CLINICAL EFFICACY AND SAFETY ENABLING SUCH PRODUCTS TO BECOME PATENTABLE ENTITIES
- INNOVATIVE APPROACHES FOR DEVELOPMENT OF NEW PROCESSES OF ISM DRUGS
- MODERN PHARMACOLOGICAL MODELS BE USED TO ENUNCIATE NEW BIOLOGICAL ACTIVITIES OF STANDARDIZED EXTRACTS WHICH BECOME PATENTABLE

Contd...

STRATEGIES FOR BIODIVERSITY CONSERVATION

- Proper Identification & Inventorying
- Geographical Information System (GIS)/Economic Mapping
- Impact of overexploitation on Supporting Habitats
- Ensuring "IN SITU " Conservation
- Assuring supply through Domestication /Agrotechnology
- Establishment of Gene Bank
- Micro and Macropropagation of Elite Plants
- Development of Low-Tech, Location specific, ECO friendly, value added Medicinal plant Products
- Ensuring free access to Medicinal Plant Resources for R&D Activities Stringent laws to prevent overexploitation
- Legal Protection to Indigenous knowledge and provision of Benefit sharing

PROTECTION OF PLANT VARIETIES

- INDIAN PATENT ACT 1970 DOES NOT ALLOW PROTECTION OF PLANT VARIETIES.
- GATT AGREEMENT PROVIDES PROTECTION OF PLANT VARIETIES EITHER BY PATENTS OR BY ANY EFFECTIVE SUI GENERIS SYSTEM OR COMBINATION THEREOF.

  PLANT VARIETY PROTECTION ACT IN U.S.

  PLANT VARIETIES AND SEED ACT IN U.K.

  (THESE COVER BREEDERS RIGHT AND FARMERS RIGHT.)

- INDIA HAS YET TO EVOLVE AN EFFECTIVE METHOD OF PLANT PROTECTION.

- **PATENTABLE NEW VARIETY APPLIES TO ANY CULTIVAR, CLONE, LINE, STOCK OR HYBRID, WHICH SATISFIES THE FOLLOWING :**

  - **NEW VARIETY MUST BE SUFFICIENTLY HOMOGENOUS, (HAVING REGARD TO PARTICULAR FEATURES OF ITS SEXUAL/VEGETATIVE REPRODUCTION).**

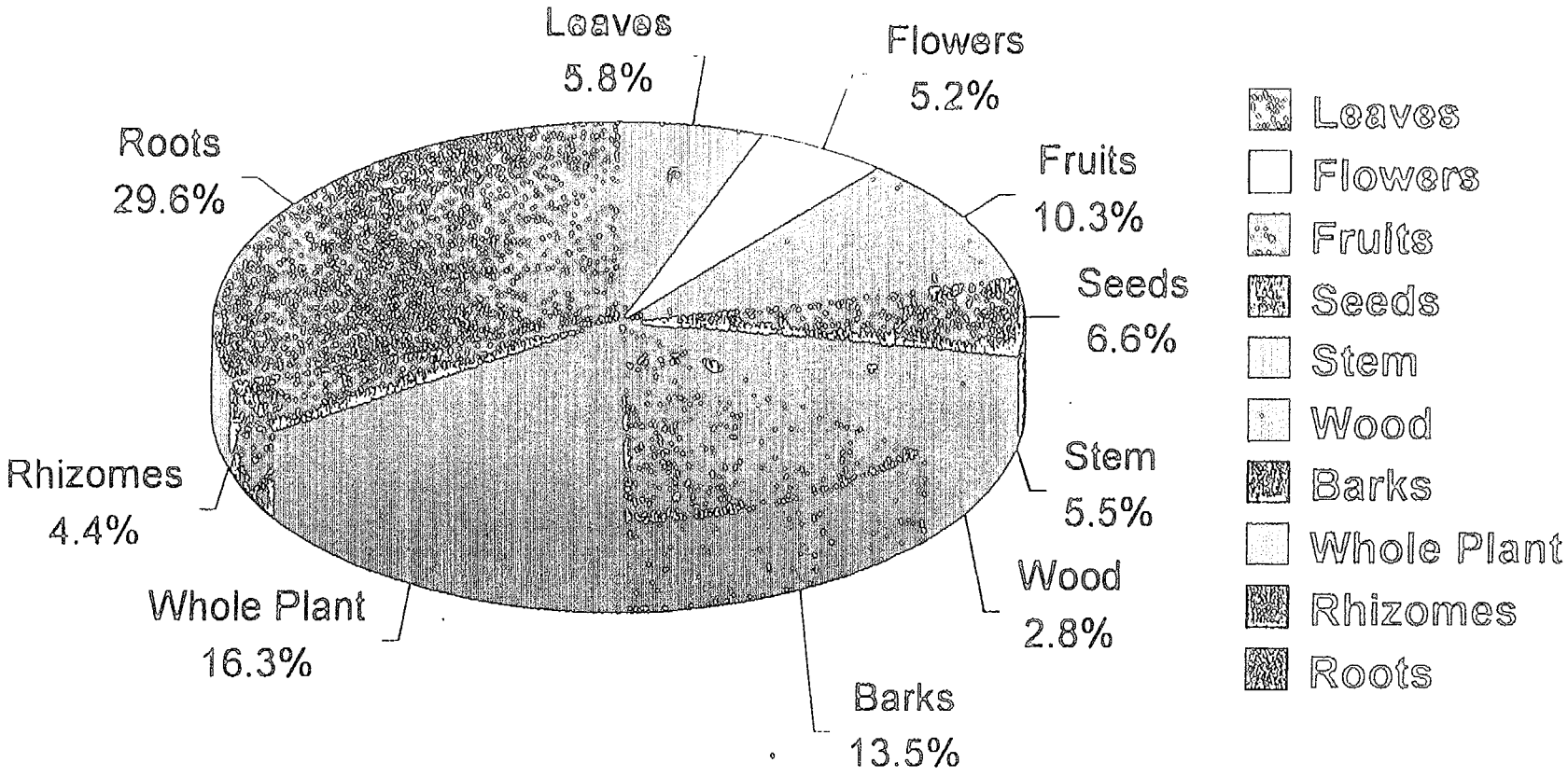
  - **NEW VARIETY SHOULD BE STABLE IN ITS ESSENTIAL CHARACTERISTICS (CHARACTERISTICS TO REMAIN UNCHANGED ON REPEATED REPRODUCTION).**

  - **NEW VARIETY SHOULD BE DISTINCT AND DISTINCTLY NAMED.**

  - **LEGAL ACTION AGAINST ANYONE WHO USES SIMILAR OR CONFUSINGLY SIMILAR NAMES.**

DESTRUCTIVE COLLECTION:

Distribution of Medicinal plants by parts used (72% destructive and 28% nondestructive)



MEDICINAL PLANTS PATENT FACT SHEET

Plants	Patents (No)	Patentee	Assignee
Blackcumin	2	Rajko D Medenica (US)	-
Bittergourd	2	(i) Sylvia Lee-Huang Philip Huang (ii) Miles C Hiffstutler Jr Gary M Steuart, MN, USA	New York University New York,
Indian mustard	14	Patented in Canada, France, China and US	Parties in Canada, France, China and US
Kumari	3	Natale, Vittori Michael Collins and two others	Wisconsin Alumni Research Foundation, USA

TEAM-CSIR

Bhuamla	4	(i) Two patents by Pinayur S Venkatesh-waran Irving Millman, Baruch (ii) Michinori Kubo Reiko Matsuda (iii) Surendra Rohatgi, India.	Fox Chase Cancer Center, Pennsylvania USA Senju Pharmaceutical Co Ltd, Japan.
Pomegranate	1	Toyohary Hazumi Takao Matsumoto and others, Japan.	None
Dudhi	1	Jeffrey J Ares, Peter D Murray and others	Procter and Gamble Co.

MEDICINAL PLANTS PATENT FACT SHEET

Plants	Patents (No)	Patentee	Assignee
Blackcumin	2	Rajko D Medenica (US)	-
Bittergourd	2	(i) Sylvia Lee-Huang Philip Huang (ii) Miles C Hiffstutler Jr Gary M Steuart, MN, USA	New York University New York,
Indian mustard	14	Patented in Canada, France, China and US	Parties in Canada, France, China and US
Kumari	3	Natale, Vittori Michael Collins and two others	Wisconsin Alumni Research Foundation, USA

TEAM-CSIR

Bhuamla	4	(i) Two patents by Pinayur S Venkatesh-waran Irving Millman, Baruch (ii) Michinori Kubo Reiko Matsuda (iii) Surendra Rohatgi, India.	Fox Chase Cancer Center, Pennsylvania USA Senju Pharmaceutical Co Ltd, Japan.
Pomegranate	1	Toyohary Hazumi Takao Matsumoto and others, Japan.	None
Dudhi	1	Jeffrey J Ares, Peter D Murray and others	Procter and Gamble Co.

RECENT TRENDS

IN NUTRICEUTICALS

Mr. Vivek Dhawan

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname : Dhawan Other name : Vivek

Title (Mr, Mrs, Dr, Professor, etc) ~~Mr, Mrs, Dr, Professor~~

Current Position : CEO-MEDICAP LIMITED

Current Place of Work : Medicap limited, 384 Soi 6, Pattana 3 Road, Bangpoo Industrial Estate, Samutprakarn 10280, Thailand

Educational profile:

Year	Place of Study	Qualification	Field of study
1979-84	New Delhi, India	BE. ENG.	Mechanical
1984-86	Carbondale, Illinois, USA	MBA	General Management

Professional Experiences :

- Project manager — Barium International
- Factory manager — Medicap Limited
- General manager — Medicap Limited / Lupin Chemicals
- Project manager — Lupin Chemicals, Thailand
- Managing director — Medicap Limited / Mega Productssltd.

Current interests :

- Reading - Philosophy / Natural / Herbal Medicine

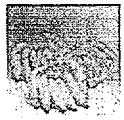
Recent Trends in Neutraceutical

Vivek Dhawan
Medicap Ltd.

Functional Foods / Health Supplements

- Nutraceuticals
- Complementary Medicine
- Alternative Medicine
- Functional Foods

Foods

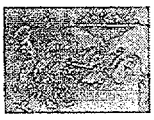


Constipation

Inflammation

Digestive

Carminative



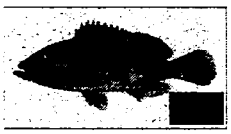
Protein

Ginger

Foods

Oyster 

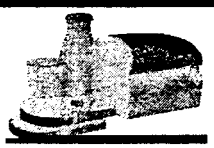
Virility



Brains & eyes

New Forms of Food

Cereals with vitamins & fibres 

Calcium enhanced milk 

Eggs fortified with DHA

Lycopene from tomatoes 

Traditional Medicines

- **Ayurveda**

Adathoda Vasica	Cough
Ashwagandha	Energy, Indian Ginseng
Bacopa	Energy & Memory
Neem	Fungicide & Contraceptive
Tamarind & Butter with Herbs	Liver Health

•Traditional Chinese Medicine

Ginseng	Tonic, Stamina
Tang Kwui (Dong Qui)	Tonic, Menstrual pain
Leng Aieng (Kao Kui)	Fever, Tonic
Lor Hung Kouy	Thirst Imbalance of Yin-Yang
Jub Leang	Thirst Imbalance of Yin-Yang

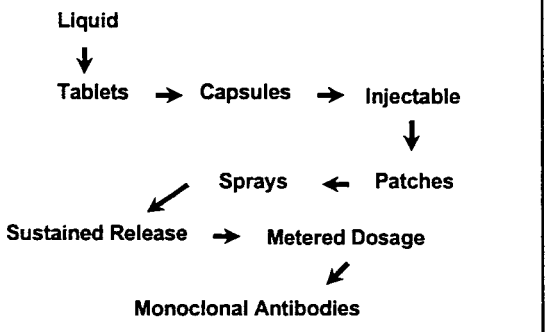
•Homeopathy

GLON. (Glonoinum +Nitroglycerine + Glyceryl trinitrate)	Headache, Hot flushes
ARNICA (Arnica mountain+ leopard's bane + Mountain tobacco + Sneezewort)	Bruises, Pain, Sprains
APIS (Apis melliifica+ Apis mellifera+ honey bee)	Restlessness, Depression

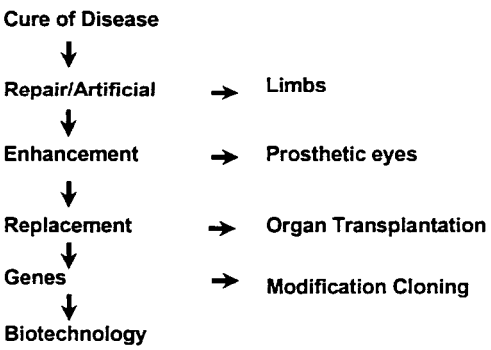
Herbal Isolate

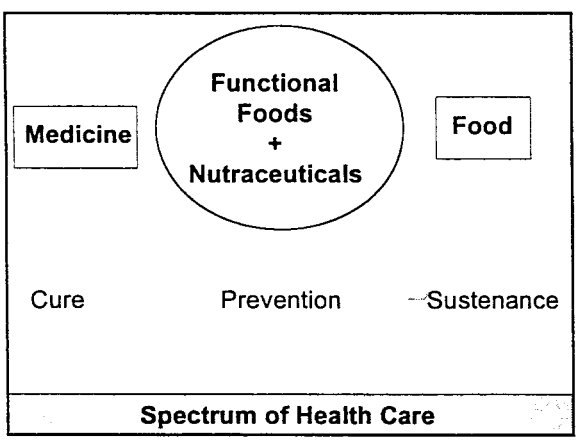
Rouwolfia Serpentina	**Reserpine**	**Cardiovascular**
Salix Alba	**Aspirin**	**Anti inflammatory**
Vinca Rosea	**Vincristin**	**Anticancer**
Digitalis Purpurea	**Digitalis**	**Cardiotonic**
Paclitaxel	**Taxol**	**Anticancer**

Dosage Development



Development in Medicine





What are we preventing?

Age & lifestyle related disorders:

- Cancer
- Heart disease
- Osteoporosis
- Prostatitis
- Infections
- Behavioural disorders
- Sleep disorders
- Sexual dysfunction

Why the interest is in this area ?

- **A large portion of population is growing old.**
- **Government is looking at ways to cut health care spending.**
- **Modern Medicines haven't provided final answers.**
- **Prevention is in fore front through food, diet, exercise, fortification & supplements.**
- **Scientific research is showing beneficial evidence for Nutraceuticals.**

A model for development

- A look at the directions emerging from the way each important player is moving
- A model for further research and development & growth

Signs of Industry Moving in This Direction

Markets

Market		*Volume*	*Growth*
US	Foods	$ 15.2 billion	12%
	Herbs	$ 3.2 billion	20%
UK		$900.0 million	6%
Germany		$ 3.5 billion	50%
France		$ 1.8 billion	26%

More and more people are turning to herbs & nutraceuticals.

INDUSTRY

New Players are expanding the Market.

- Boehringer Ingleheim bought Pharmaton.
- AHP buys Solgar vitamins.
- Nutricia bought Vitamax & Efamol / Rex-II / GNC
- SKB → Abetiie in Germany.
- Bayer launched new herbal range.
- Corn Agro, Kellog invest in functional foods business

Industry - NEW DIRECTION

- Moving towards a Pharmaceutical GMP Standard.
- Scientific & informed use of product.
- Quality Standards for raw materials & finished products.
- Research and development in stability, efficacy & quality.
- Self control, restraint in areas of claim.

Industry: Model for future

- **Invest in international standard facilities.**
- **Develop products which can benefit general health of public.**
- **Create international brands with clinical research & documentation.**

PRODUCTS

- In Germany Prescription for St.John's Wort are more than for Prozac.
- *Ginkgo biloba* is largest selling product in Germany.
- Cod Liver Oil outsells any other product in UK.
- Evening Primrose Oil is the single largest product used for PMS.

NEW PRODUCTS

- **Phosphatidyl Serine for memory.**
- **Red Clover for PMS/HRT.**
- **Soy Isoflavones for HRT/osteoporosis.**
- **Grape seed extract for cardiovascular diseases.**
- **Alpha Lipoic Acid as antioxidant.**
- **Benecol : J&Js Cholesterol lowering margarine.**
- **Olestra : Substituting vegetable oil.**

RESEARCH - STATUS

- CSIRO in Australia - Red Clover (Promensil).
- Scandinavian Universities & CSIRO - Hi-DHA for infant brain development.
- US FDA - $ 5 million funding for St. John's wort trial.
- NIH establishes Division of Alternative Medicine.
- CSIRO India - Bacopa in memory loss.
- Bastyr University: first university for herbal products in western world.
- Convance drug research - 2 Chinese herbs being developed as medicines.

Universities: Catalysts of new research

- Help in product development, analysis, formulation & stability.
- New products from local raw material.
- Developing existing products by promoting plantations & extraction facilities in country.
- Clinical research in new products & existing products.

Research & Development

Identification:

Plants
Herbs
Ingredients
Foods

By usage, ancient knowledge previous research

Research & Development

- **Growing herbs/Plants**
  - **Agricultural research**
  - **Quality parameters**
  - **Standards and procedures for**
    - Harvesting
    - Use of insecticides/fungicides
    - Testing facilities Growing herbs

Research & Development

- Processing technology
- Pilot scales and commercial up scales
- Analytical methods
- Clinical studies and efficacy

Research & Development

- Formulation Development
  - Dosage form
    - Canned/drinks.Capsules/Tablets etc.
  - Dosages
  - Palatability: Odour, taste
  - Efficacy in formulation
  - Stability
- Manufacturing standards:
  - GMP, HACCP guidelines

Regulatory Environment

New regulation being introduced:

- GMP guide lines.
- Allowing access to products proven safe & have known "function".
- Functional & health claims being allowed.
- Control on advertising to build credibility.
- More harmony between countries is expected in years to come.

Regulatory environment for future

- Open the procedures of registration to meet international criteria.
- Require GMP/HACCP international standards for all manufacturers.
- Regulate claims & increase access to correct knowledge.

Financial Institution

- More funding through Venture Capital available now than ever before.
- More companies listed in stock market.

Financial Institutions In Development

Support the industry through:

- Capital
- Long term borrowings
- Privileges
- Assistance

This is a very great opportunities for all of us

- The Governments bodies involved in Regulating these products
- Universities directing the research
- Industry
- Financial Institutions

To work together in building a tomorrow.

'An Industry - A business of the future which will help to improve the health of mankind.'

GOOD CLINICAL PRACTICE OF HERBAL MEDICINAL PRODUCTS

Professor Sornchai Looareesuvan

Guest Lecturer

Please kindly complete this form or provide us your biography

Surname :_____Looareesuwan_____Other name :_____Sornchai_____

Title (Mr, Mrs, Dr, Professor, etc) _____Professor_____

Current Position :_____Dean_____

Current Place of Work :____Faculty of Tropical Medicine, Mahidol Universiry_____

Educational profile:

Year	Place of Study	Qualification	Field of study
1974	Faculty of medicine, SIriraj Hospital	M.D.	
1978	Mahidol University	D.T.M. H.	
1978	Siriraj Hospital	Diplomat Thai Board in General Medicine	

Professional Experiences :

___1974-1975____ Rotating intern, Siriraj Hospital_____

___1976-1978____ Resident training in General Medicine, Siriraj Hospital _____

___1979-present__Department of Clinical Tropical Medicine, Faculty of Tropical Medicine

___1981-1982_____Welcome Research Fellow, Nuffield Dept. of Clinical Med, U of oxford_

___1984 _____FRCP(I)_____

___1995 _____Factm (Fellow of the Australian College of Trop. Med. James Cock U.

_____of N. Queenland Australia)_____

___1996-present__Director, Seamed Tropmed / Thailand_____

___1998-present__Secretary General, Seamed Tropmed Network_____

Current interests :

___- Malaria_____

___- Snake Bite_____etc._____

Clinical Trial and Good Clinical Practice

Sornchai Looareesuwan
Dean, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

The aim of clinical trial is to study for an answer of safety and efficacy of the drugs. Therefore, a prospective and control arm is needed. Firstly, a proposal, which defines a purpose of the trial, design, conduction, analysis and conclusion, has to be written. It is a general rule that one must review basic biological data as well as data in animal studies before performing a clinical trial.

In general, clinical trial is divided into 4 phases. Phase I (clinical/pharmacological study in healthy volunteers) is usually non-blind performed in a clinical research center or specialty trained clinical pharmacologist. Its aim is to determine safety and clinical pharmacology with single or repeat dosage in an open study design.

Phase II (trial) is aimed to determine clinical efficacy, safety and side effects of an investigational drug in patients. It is normally done as a single blind design in a limited number of subjects in special research centers.

Phase III (clinical trial in a large group of patients) is aimed to confirm efficacy and occurrence of adverse events. It is normally done as a double blind or comparative design with a standard regimen in a large group of patients. This phase can be performed in special group of patients (eg. pediatric, pregnant, geriatric) and can also be performed as multicenter studies. If the trial succeeds this phase, the investigational drug is normally granted registration from regulatory authority for using in public patients.

Phase IV (continuing or observation after marketing) is a post marketing surveillance study. It is aimed to find rare adverse events that cannot be detected in phase II or III trials (incident of adverse event < 1:10,000 population). Moreover, it aims to find evidences on drug interaction, compliance and pharmacogenetic when the study drug has been extensively used in public.

To perform a clinical trial, a scientific research proposal is needed. This proposal must include the primary and secondary endpoints of the study, why (problem), how to be done (subjects, inclusion and exclusion criteria), what (variables, ethics, costs) and when (to start, analysis, and publication).

There are many types of protocols and designs (prospective or retrospective studies). They need planning, organizing, executing, evaluating, and finally publishing the results.

The planning of a research project includes the proposal of the study, known problems, design to be used, and the method of choosing subjects. Planning also includes the intervention in the study, the method of defining and measuring variables, data to be collected and analyzed, the possible ethical problems, arrangements to be made, the starting time, and the costs of the project. Types of

investigations include place (field, hospital, laboratory), time (retrospective, concurrent, or experimental) and purposes (epidemiological, evaluative, surveillance).

Study protocol operation must include inclusion and exclusion criteria, study drug allocation (random or open) and discontinuation criteria. In addition, all these data (control, randomization, blind or unblind, sample size) should be specified in the protocol in order to reduce clinical trial errors. Any clinical trial must answer a specific scientific question.

Developing a good protocol is a difficult part of a project or it must be performed according to Good Clinical Practice (GCP). GCP is an international ethical and scientific quality standard for designing, conduction, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are creditable.

The objective of GCP must facilitate the mutual acceptance of clinical data by the regulatory authorities. The principles of GCP include: ethic, risk and benefit, rights, safety and well-being of the subjects. Available non clinical and clinical information on an investigational product, scientifically sound and clearly described in protocol, prior to be approved by institutional review board (IRB). Before a a trial starts, it is the responsibility of qualified physicians to explain and clearly inform subjects about the risks/benefits. Study subjects should also be informed consent procedure and free withdrawal from the study at any time, data/records handled accurately for interpretation in a confidential manner. The investigational products manufactured/ handled and stored in accordance with good manufacturing practice (GMP). Unless these points met, GCP cannot be obtained.

GUIDELINE FOR GOOD CLINICAL PRACTICE (GCP)

Somchai Looareesuwan
Dean, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

1) Principles of GCP

1.1 Clinical trial should be conducted in accordance with the ethical principles that have their origin in the Declarationof Helsinki.

1.2 Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.

1.3 The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

1.4 The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.

1.5 Clinical trials should be scientifically sound, and described in a clear, detailed protocol.

1.6 A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB) approval.

1.7 The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician.

1.8 Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).

1.9 Freely given informed consent should be obtained from every subject prior to clinical trial participation.

1.10 All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.

1.11 The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

1.12 Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.

1.13 Systems with procedures that assure the quality of every aspect of the trial should be implemented.

2) Institutional Review Board (IRB)

2.1 Responsibilities

An IRB should safeguard the rights, safety, and well-being of all trial subjects. Special attention should be paid to trials that may include vulnerable subjects.

2.2 Composition, Functions and Operations

The IRB should consist of a reasonable number of members, who collectively have the qualifications and experience to review and evaluate the science, medical aspects, and ethics of the proposed trial. It is recommended that the IRB should include:

(a) At least five members.
(b) At least one member whose primary area of interest is in a nonscientific area.
(c) At least one member who is independent of the institution/trial site.

2.3 Procedures

The IRB should establish, document in writing,

2.4 Records

The IRB should retain all relevant records (e.g., written procedures, membership lists, lists of occupations/affiliations of members, submitted documents, minutes of meetings, and correspondence) for a period of at least 3 years after completion of the trial and make them available upon request from the regulatory authority(ies).

3) Investigator

3.1 Qualifications and Agreements

The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should proved evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB, and/or the regulatory authority(ies).

3.2 Adequate Resources

The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3.3 Medical Care of Trial Subjects

A qualified physician should be responsible for all trial-related medical decisions.

3.4 Communication with IRB

During the trial the investigator should provide to the IRB all documents subject to review.

3.5 Compliance with Protocol

The investigator should conduct the trial in compliance with the protocol agreed to by the sponsor.

3.6 Investigational Product(s)

Responsibility for investigational product(s) accountability at the trial site(s) rests with the investigator.

3.7 Randomization Procedures and Unblinding

The investigator should follow the trial randomization procedures.

3.8 Informed Consent of Trial Subjects

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.

3.9 Records and Reports

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

3.10 Progress Reports

The investigator should submit written summaries of the trial status to the IRB annually, or more frequently, if requested by the IRB.

3.11 Safety Reporting

All serious adverse events (SAEs) should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subject rather than by the subjects' names, personal identification number, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB

3.12 Premature Termination or Suspension of a Trial

In the trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority(ies).

3.13 Final Report(s) by Investigator

Upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/ institution should provide the IRB with a summary of the trial's outcome, and the regulatory authority(ies) with any reports required.

4) Sponsor

4.1 Quality Assurance and Quality Control

The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

4.2 Contract Research Organization (CRO)

A sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data

always resides with the sponsor. The CRO should implement quality assurance and quality control.

4.3 Medical Expertise

The sponsor should designate appropriately qualified medical personnel who will be readily available to advise on trial related medical questions or problems. If necessary, outside consultant(s) may be appointed for this purpose.

4.4 Trial Design

The sponsor should utilize qualified individuals (e.g. biostatisticians, clinical pharmacologists, and physicians) as appropriate, throughout all stages of the trial process, from designing the protocol and CRFs and planning the analyses to analyzing and preparing interim and final clinical trial reports.

4.5 Trial Management, Data Handling, and Record Keeping

The sponsor should utilize appropriately qualified individuals to supervise the overall conduct of the trial, to handle the data, to verity the data, to conduct the statistical analyses, and to prepare the trial reports.

4.6 Investigator Selection

The sponsor is responsible for selecting the investigator(s)/institution(s). Each investigator should be qualified by training and experience and should have adequate resources to properly conduct the trial for which the investigator is selected. If organization of a coordinating committee and/or selection of coordinating investigator(s) are to be utilized in multicentre trials, their organization and/or selection are the sponsor's responsibility.

4.7 Allocation of Duties and Functions

Prior to initiating a trial, the sponsor should define, establish, and allocate all trial-related duties and functions.

4.8 Compensation to Subjects and Investigators

If required by the applicable regulatory requirement(s), the sponsor should provide insurance or should indemnify (legal and financial coverage) the investigator/the institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence.

4.9 Financing

The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/ institution.

4.10 Notification/Submission to Regulatory Authority(ies)

Before initiating the clinical trial(s), the sponsor (or the sponsor and the investigator, if required by the applicable regulatory requirement (s) should submit any required application(s) to the appropriate authority(ies) for review, acceptance, and/or permission (as required by the applicable regulatory requirement(s) to begin the trial(s). Any notification/submission should be dated and contain sufficient information to identify the protocol.

4.11 Confirmation of Review by IRB

The sponsor should obtain from the investigator/institution documentation and dates of any IRB reapprovals/re-evaluations with favourable opinion, and of any withdrawals or suspensions of approval/favourable opinion

4.12 Information on Investigational Product(s)

When planning trials, the sponsor should ensure that sufficient safety and efficacy data from nonclinical studies and/or clinical trials are available to support human exposure by the route, at the dosages, for the duration, and in the trial population to be studied.

4.13 Manufacturing, Packaging, Labeling, and Coding Investigational Product(s).

The sponsor should ensure that the investigational product(s) (including active comparator(s) and placebo, if applicable) is characterized as appropriate to the stage of development of the product(s), is manufactured in accordance with any applicable GMP, and is coded and labeled in a manner that protects the blinding, if applicable. In addition, the labeling should comply with applicable regulatory requirement(s)

4.14 Supplying and Handing Investigational Product9s)

The sponsor is responsible for supplying the investigator(s)/institution(s) with the investigational product(s).

4.15 Record Access

The sponsor should ensure that it is spedified in the protocol or other written agreement that the investigator(s) institution(s) provide direct access to source data/documents for trial-related monitoring, audits, IRB review, and regulatory inspection.

4.16 Safety Information

The sponsor is responsible for the ongoing safety evaluation of the investigational product(s)

4.17 Adverse Drug Reaction Reporting

The sponsor should expedite the reporting to all concerned investigator (s)/institutions(s), to the IRB(s), where required, and to the regulatory authority(ies) of all adverse drug reactions (ADRs) that are both serious and unexpected.

4.18 Monitoring

The purposes of trial monitoring are to verity that:

(a) The rights and well-being of human subjects are protected.

(b) The reported trial data are accurate, complete, and verifiable from source documents.

(c) The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

4.19 Audit

If or when sponsors perform audits, as part of implementing quality assurance, they should consider:

4.20 Noncompliance

Noncompliance with the protocol, SOPs, GCP, and /or applicable regulatory requirement(s) by an investigator/institution, or by member(s) of the sponsor's staff should lead to prompt action by the sponsor to secure compliance.

4.21 Premature Termination or Suspension of a Trial

If a trial is prematurely terminated or suspended, the sponsor should promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB should also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

4.22 Clinical Trial/Study Reports

Whether the trial is completed or prematurely terminated, the sponsor should ensure that the clinical trial reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor should also ensure that the clinical trial reports in marketing applications meet the standards of the Guideline for Structure and Content of Clinical Study Reports. (NOTE: The Guideline for Structure and Content of Clinical Study Reports specifies that abbreviate study reports may be acceptable in certain cases.)

4.23 Multicentre Trials

All investigators conduct the trial in strict compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies), and given approval/ favourable opinion by the IRB.

5) Clinical Trial Protocol and Protocol Amendment(s)

5.1 General Information

Protocol title, protocol identifying number, and date. Any amendment(s) should also bear the amendment number(s) and date(s).

5.2 Background Information

Name and description of the investigational product(s).

5.3 Trial Objectives and Purpose

A detailed description of the objectives and the purpose of the trial

5.4 Trial Design

The scientific integrity of the trial and the credibility of the data from the trial depend substantially on the trial design. A description of the trial design, should include:

5.5 Selection and Withdrawal of Subjects
Subject inclusion criteria, subject exclusion criteria.

5.6 Treatment of Subjects
The treatment9s) to be administered, including the name9s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) for subjects for each investigational product treatment/trial treatment group/ arm of the trial.

5.7 Assessment of Efficacy
Specification of the efficacy parameters. Methods and timing for assessing, recording, and analyzing of efficacy parameters.

5.8 Assessment of Safety
Specification of safety parameters. The methods and timing for assessing, recording, and analyzing safety parameters.

5.9 Statistics
A description of the statistical methods to be employed, including timing of any planned interim analysis(ses).

5.10 Direct Access to Source Data/Documents
The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB review, and regulatory inspection9s), providing direct access to source data/documents.

5.11 Quality Control and Quality Assurance
Description of ethical considerations relating to the trial/

5.12 Ethics
Description of ethical considerations relating to the trial.

5.13 Data Handling and Record Keeping

5.14 Financing and Insurance
Financing and insurance of not addresses in a separate agreement.

5.15 Publication Policy
Publication policy, if not addressed in a separate agreement.

5.16 Supplements
(NOTE: Since the protocol and the clinical trial/study report are closely related, further relevant information can be found in the ICH Guideline for Structure and Content of Clinical Study Reports.)

6) Investigator's Brochure

6.1 Introduction

The Investigator's Brochure (IB) is a complilation of the clinical and nonlcinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects. Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, may key features of the protocol, such as the dose, dose frequency/ interval, methods of administration: and safety monitoring procedures.

6.2 General Considerations

The IB should include:

Title Page
Confidentiality Statement

6.3 Contents of the Investigator's Brochure

A description should be provided of the investigational product substance(s) (including the chemical and/or structural formula(e)), and a brief summary should be given of the relevant physical, chemical, and pharmaceutical properties.

7) Essential documents for the conduct of a clinical trial

Essential Documents are those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor and monitor with the standards of Good Clinical Practice (GCP) and with all applicable regulatory requirements.

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Research note

A comparison of three different dihydroartemisinin formulations for the treatment of acute uncomplicated falciparum malaria in Thailand

P. Wilairatana[a], P. Chanthavanich[b], P. Singhasivanon[c], S. Treeprasertsuk[c], S. Krudsood[c], K. Chalermrut[d], C. Phisalaphong[e], K. Kraisintu[e], S. Looareesuwan[a,*]

[a]*Department of Clinical Tropical Medicine,* [b]*Department of Tropical Pediatrics,* [c]*Department of Tropical Hygiene,* [d]*Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine,* [e]*Government Pharmaceutical Organization, Bangkok 10400, Thailand*

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Abstract

We compared the safety and efficacy of three formulations of dihydroartemisinin for the treatment of acute uncomplicated falciparum malaria in patients who received a total dose of 600 mg dihydroartemisinin over 5 days. The first group was treated by dihydroartemisinin produced and formulated in the People's Republic of China, the second group was treated by dihydroartemisinin produced in Vietnam but formulated by the Government Pharmaceutical Organization of Thailand and the third group was treated by dihydroartemisinin produced and formulated by the Government Pharmaceutical Organization of Thailand. All patients were admitted to hospital to evaluate safety and efficacy for a total of 28 days. By the third day of treatment, most patients were blood-smear negative for parasites and none had serious adverse effects. Minor symptoms such as nausea, dizziness and headache were similar in the three groups and disappeared after 3 days of treatment. One-hundred and thirty-three patients completed the 28-day follow-up period. The cure rates of groups I, II and III were 80%, 85% and 92%, respectively ($P > 0.02$). There were no significant differences in fever clearance or parasite clearance among the three groups. We conclude that the three formulations of dihydroartemisinin produced and formulated in different countries were safe and effective in treating uncomplicated falciparum malaria acquired in Thailand. © 1998 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Keywords: Falciparum malaria; Treatment and dihydroartemisinin; Antimalarial chemotherapy; Artemisia alkaloids; Qinghausu

Treating *Plasmodium falciparum* malaria in Southeast Asia is increasingly difficult because of the rapid increase in multidrug-resistance in parasite populations in this area, particularly in Thailand. Resistance to most antimalarial therapies is well-documented [1]. Quinine plus tetracycline for 7 days remains the standard regimen for falciparum malaria in Thailand, with cure rates of 90–98% when drug administration is supervised [2, 3]. However, both drugs have a short half-life (6–8 h) that

*Corresponding author. Present address: Faculty of Tropical Medicine, 420 6 Rajavithee Road, Bangkok 10400, Thailand. Fax: (662) 247-1688; e-mail: tmslr@mucc.mahidol.ac.th.

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necessitates frequent dosing, and quinine causes significant side effects. Thus, treatment with quinine and tetracycline is associated with poor patient compliance, especially in the out-patient setting. Mefloquine has been used as a single dose, but the cure rate for the standard regimen of 15 mg kg^{-1} fell from 98% in the years 1983–1986 [4, 5] to 71% in 1990 [6]. Even doubling the dose to 25 mg kg^{-1} does not guarantee cure [7–11].

Dihydroartemisinin, an artemisinin derivative, has been evaluated in clinical trials in Thailand for the treatment of falciparum malaria during 1994–1995 [11]. Dihydroartemisinin is a potent antimalarial drug that can reduce parasitaemia by 90% within 24 h of administration [11, 12] and in our previous study gave a 90% cure rate [11]. All the artemisinin derivatives are metabolised rapidly to the active metabolite dihydroartemisinin [13]. The use of dihydroartemisinin instead of the substitute compounds (e.g. artesunate or artemether) has advantages. The drug is easy to produce with less synthetic steps and, thus, a lower cost. However, there are some doubts over the source of raw material for production and the formulations made, so we prospectively compared the efficacy and safety of three different sources and formulations of dihydroartemisinin in patients with uncomplicated falciparum malaria acquired in Thailand.

Patients admitted to the Bangkok Hospital for Tropical Diseases, Thailand, between January 1996 and July 1997, were accepted into the study if they were diagnosed as having acute, uncomplicated falciparum malaria with 100–250 000 parasites per μl of blood. Patients enrolled in the study were 15–65 years old, weighed at least 40 kg, gave informed consent, and agreed to remain in hospital for a total of 28 days. Reasons for exclusion included pregnancy, severe or complicated malaria [14], or a history of antimalarial ingestion in the previous 2 weeks. The Ethics Committee of the Mahidol University, Bangkok, Thailand, approved the study protocol.

Patients were assigned to one of three treatment regimens. Patients in group I received a dose of 200 mg of oral dihydroartemisinin (Cotexin® 20 mg tablet, Cotec, Beijing, People's Republic of China) on the first day of treatment and then 100 mg of dihydroartemisinin daily for 4 consecutive days for a total dose of 600 mg. Group II received a dose of 200 mg of oral dihydroartemisinin (Vietnam product, formulated by the Government Pharmaceutical Organization of Thailand) on the first day of treatment and then 100 mg of dihydroartemisinin daily for 4 consecutive days for a total dose of 600 mg. Group III received a dose of 200 mg of oral dihydroartemisinin (Thailand product, formulated by the Government Pharmaceutical Organization) on the first day and then 100 mg of dihydroartemisinin daily for 4 consecutive days for a total dose of 600 mg.

The sample sizes of 74, 23 and 60 patients for groups I, II and III, respectively, were the result of the limitation of drug supply. The study was performed as a sequential trial. However, the analysis at the end of the study was done by one of the co-authors who did not know the study design and test drugs used. Analyses using ANOVA compared the mean cure rate, fever and parasite clearance times in the three groups.

Routine haematology and biochemistry evaluations were performed prior to treatment and repeated on days 7, 14, 21 and 28. Thick blood films were examined every 12 h for malaria parasites until negative, and then were performed daily until discharge. Parasite counts per μl were determined by counting the number of asexual parasites per 200 white blood cells in thick films or per 1000 red blood cells in thin films. Blood films were considered negative if no parasites were seen in 200 oil immersion fields in a thick blood film. Cure was defined as the absence of recrudescent falciparum malaria during the 28-day study period [15]. Parasite clearance time was defined as the time from the start of treatment until blood films were negative and remained negative for the next 24 h. Fifty per cent or 90% parasite reduction times were defined as the time calculated from the start of treatment until the parasitaemia dropped by either 50% or 90% of the initial value, respectively. Fever clearance time was defined as the time from the start of treatment until the oral temperature dropped to $<37.5^{\circ}$C and remained below this mark for at least 48 h. Oral temperature, pulse and respiratory rates were measured every 4 h and blood pressure was measured once a day. Monitoring for signs and symptoms of malaria was performed daily for the first 7 days of hos-

Table 1
Clinical and laboratory characteristics of the three study groups before treatment

	Group I (n = 74)	Group II (n = 23)	Group III (n = 60)
Male female	52 22	14 9	50 10
Age (years)			
Mean (±S.D.)	26.8 (10.2)	24.3 (10.6)	27.8 (1.0)
Range	15–59	15–63	15–55
Height (cm)			
Mean (±S.D.)	160.4 (8.6)	158.6 (8.0)	162.1 (7.6)
Weight (kg)			
Mean (±S.D.)	52.9 (10.3)	48.7 (8.4)	55.2 (11.0)
Fever [mean (±S.D.)]			
Duration before admission (days)	4.2 (3.2)	4.5 (2.6)	4.3 (3.2)
Temperature before treatment (°C)	38.0 (0.7)	38.0 (0.7)	37.9 (0.9)
Number of patients with (%):			
Splenomegaly	15 (20)	2 (9)	4 (7)
Hepatomegaly	28 (38)	7 (30)	17 (28)
G-6-PD deficiency	2 (3)	4 (17)	6 (10)
Urine positive for 4-aminoquinolines	12 (16)	7 (30)	23 (38)
First malarial attack	47 (63)	19 (83)	36 (60)
Geometric mean parasite counts (per μl)	10 651	8254	7406
Range	195–146 520	124–215 100	189–131 040
Laboratory data [mean (±S.D.)]			
Packed cell volume (%)	33.5 (7.9)	32.3 (10.2)	35.1 (7.5)
WBC counts (per μl)	6365 (1977)	6663 (2860)	6138 (2069)
BUN (mmol l^{-1})	25.9 (10.7)	14.8 (6.4)	12.7 (4.9)
Serum creatinine (μmol l^{-1})	0.97 (0.25)	0.91 (0.14)	0.93 (0.17)
Total bilirubin (μmol l^{-1})	1.4 (1.3)	2.6 (3.3)	1.5 (1.3)
Serum AST (i.u. × 10^3 l^{-1})	39.0 (29.2)	71.7 (70.2)	48.9 (42.8)
Serum ALT (i.u. × 10^3 l^{-1})	39.5 (48.5)	56.3 (42.3)	50.4 (55.3)
Albumin (mg dl^{-1})	4.1 (0.5)	3.7 (0.6)	4.0 (0.5)

WBC = white blood cell; BUN = blood urea nitrogen (mmol l^{-1}); AST, ALT = aspartate and alanine aminotransferases (i.u. × 10^3 l^{-1}).

pitalisation and weekly thereafter. Detailed neurological examinations were performed daily during treatment and weekly later on. A standardised questionnaire was used to assess side effects.

The laboratory and clinical characteristics of the three groups were comparable before treatment (Table 1). By history, the majority of patients, 65% (102/157), were experiencing their first malaria attack, and most had contracted the infection on the Thai–Myanmar border. Twelve, 7 and 23 patients in groups I, II and III, respectively, had trace amounts of chloroquine in the urine on admission.

All patients improved clinically within 1–3 days after treatment was started. Twenty-four patients withdrew from the study for reasons unrelated to drug treatment or side effects (9, 3 and 12 patients from groups I, II and III, respectively). All were parasite-free and asymptomatic on withdrawal. The remaining 133 patients who completed the 28-day follow-up were analysed for drug efficacy.

The cure rates, number of subsequent recrudescences of *P. falciparum* malaria during the 28-day follow-up and the median time to recrudescence of the infections are detailed in Table 2. Patients with recrudescence were cured with a 7-day course of quinine plus tetracycline. There were no significant differences in either parasite clearance time or fever clearance time among patients of groups I, II and III ($P > 0.05$). The mean parasite reduction times by 50% and 90% were, respectively, 11.5 and

Table 2
Therapeutic responses

	Group I (n = 74)	Group II (n = 23)	Group III (n = 60)
No. of patients with 28-day follow-up	65	20	48
No. of withdrawals	9	3	12
Resistance type-I response	13	3	4
Cure rate at 28 days (%)	52 65 (80)	17/20 (85)	44 48 (92)
Recrudescence on day			
Median (range)	24 (13–28)	18 (18–27)	18 (14–22)
Fever clearance time (h)			
Mean (±S.D.)	42.6 (41.2)	39.4 (26.6)	35.0 (22.3)
Range	4–296	4–110	12–81
Parasite clearance time (PCT) (h)			
PCT 100%			
Mean (±S.D.)	46.2 (11.7)	51.1 (23.4)	43.4 (14.1)
Range	24–72	4–127	12–81
PCT 90%			
Mean (±S.D.)	18.6 (7.6)	22.6 (14.9)	17.7 (8.0)
Range	6–42	8–84	6–51
PCT 50%			
Mean (±S.D.)	11.5 (6.1)	13.6 (12.1)	10.2 (6.5)
Range	5–30	6–59	5–27
Patients with *P. vivax*			
No. of patients with *P. vivax* (%)	10 (13)	2 (8)	4 (6)
Median day of appearance (day)	19	24	22
Range (day)	18–28	24	14–28

18.6 h for group I, 13.6 and 22.6 h for group II, and 10.2 and 17.7 h for group III (Fig. 1).

During the follow-up period, 16 patients (10 in group I, two in group II and four in group III) had blood smears positive for asexual forms of *Plasmodium vivax* between days 14 and 28 of hospitalisation. All patients were treated with a single low-dose chloroquine (450 mg base) on the day of diagnosis to suppress infection. After the 28-day follow-up period for the dihydroartemisinin study, patients with vivax malaria were treated with 1500 mg of chloroquine over 3 days and 15 mg of primaquine daily for 14 days as a standard treatment for vivax malaria.

Patients in the three treatment groups tolerated the drug well and there were no differences among the groups. No major adverse effects presented. Minor complaints consisted of headache (21%), dizziness (20%) and nausea (18%). Most of these complaints were recorded on the first 2 days of treatment and generally coincided with high fever. There was no evidence for neurotoxicity in any patient. Twelve patients were G-6-PD deficient, but there was no clinically significant haemolysis. One patient in group I, three patients in group II and three patients in group III had elevated serum transaminase(s) or total bilirubin levels prior to treatment. All values returned to normal within 1–2 weeks.

Dihydroartemisinin has been used in the treatment of multidrug-resistant falciparum malaria in China. The original formulation consisted of 20-mg tablets (Cotexin®, Beijing, China) and is licensed for use in China. Recently, three new formulations made in Vietnam, Thailand and The Netherlands have become available for trial. However, despite the fact that millions of patients have been treated with artemisinin derivatives, dosage regimens have remained empiric. Pharmacokinetic data are scarce because measurement of these compounds in plasma or serum is difficult and expensive [23].

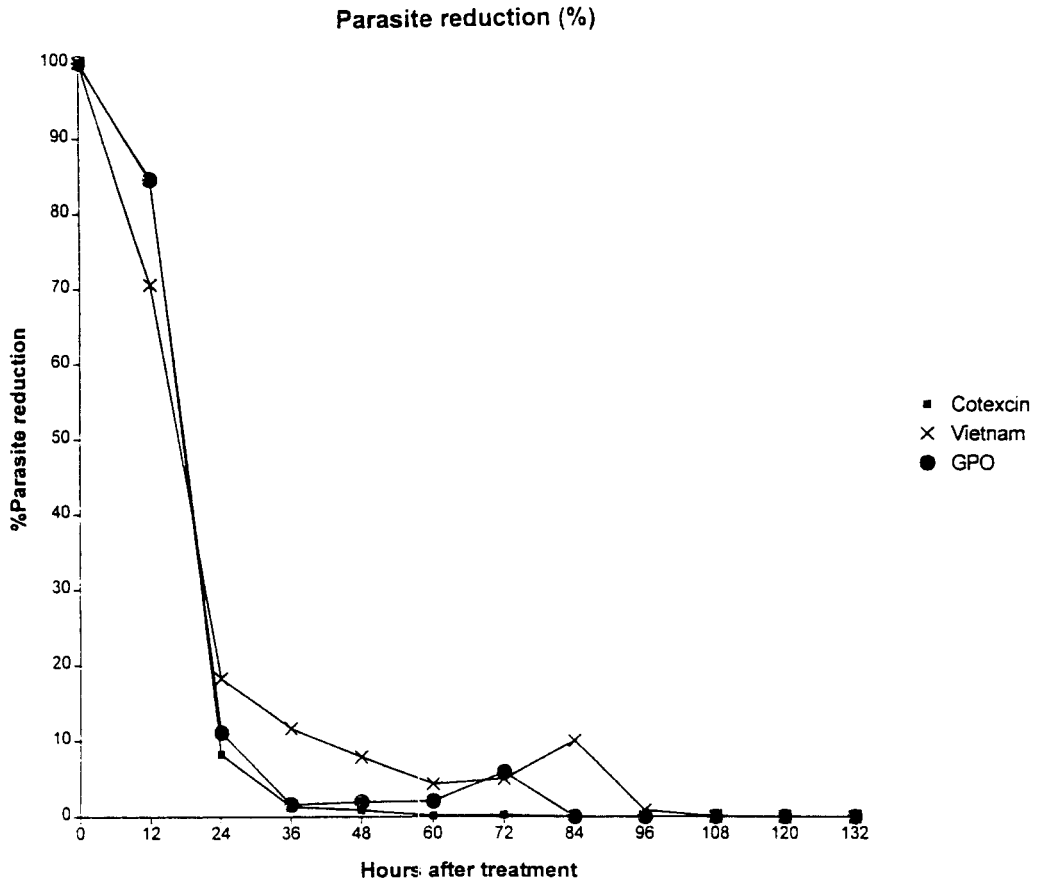

Fig. 1.

Most of the patients in this study improved clinically and were parasite negative on the blood smear by the third day of treatment with dihydroartemisinin. This observation is encouraging, but raises the concern that self-treating patients might stop the therapy earlier than necessary for complete cure and increases the probability of recrudescence. There is no documented artemisinin resistance in falciparum malaria and we have previously reported that the minimum inhibitory concentration did not show differences between acute and recrudescent isolates [9, 18, 19]. Because of the usefulness of artemisinin derivatives in severe and multidrug-resistant malaria, the availability of these drugs should be tightly regulated and they should not be given prophylactically.

The delayed appearance of *P. vivax* in 16 patients was not unexpected, because dihydroartemisinin is not effective against the hypnozoite of *P. vivax* malaria and has a short half-life. In our experience, one-third of falciparum patients treated with a short-acting antimalarial drug, such as quinine, developed vivax malaria within 2 months after the treatment of *P. falciparum* was given [27]. The low-dose chloroquine (450 mg base single dose), treatment of *P. vivax*, would have had no effects on the evaluation of dihydroartemisinin efficacy since *P. falciparum* isolates from Thailand are highly resistant to chloroquine [1].

In this study, tablet formulations of dihydroartemisinin from three different sources, in a total dose of 600 mg given over 5 days, were proved effective, safe and well tolerated, and gave cure rates of 80–92% in uncomplicated falciparum malaria. Since dihydroartemisinin is easy to make and inexpensive, it may prove useful in uncomplicated malaria acquired in multidrug-resistant areas such as Thailand.

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ANNEXE B

COUNTRY REPORTS

COUNTRY REPORT

COUNTRY: Bhutan

NAME: Mr. Phurpa Wangchuk

Mr. Phuntsho Wangchuk
Pharmaceutical and Research Unit
ITMS. Health Dept. Thimphu

Bhutan

Background

Bhutan, formerly known as 'Menjong'-the land of medicinal plants, is a small mountainous country with approximately 46,500 square kilometers nestled between India and China.

The population is 600,000. The state religion is Mahayana Buddhism. The country has been declared as one of the world's ten most global 'hotspots' of bio-diversity with 72% forest still intact.

More than 600 medicinal plants are identified and has potential to increase.

Development Perspective

As articulated by His Majesty, the country's Central Development Concept is "Maximizing Gross National Happiness rather than GDP".

Among many development programs and strategies, Health and Education is one of the important primary priority milestones. The Government provides free Health care and education to the public.

The traditional medicine was known as early as 16th century. The allopathic medicine found its root and geared only after 1960s. Now, both Traditional and Allopathic Medical Services are significantly involved in the primary health care. The government's policy is to integrate the two systems and disseminate the best hybrid for the welfare of the people.

The regulatory and spread body of traditional medicine practices is "The Institute of Traditional medicine Services".

INSTITUTE OF TRADITIONAL MEDICINE SERVICES

The Institute has undergone a long history of metamorphosis since its first establishment in 1967. For effective management and administration, the Institute was divided into three Units in 1998.

- Indigenous Hospitals to provide traditional medical care services to the public.
- The Institute provides human resource development.
- Pharmaceutical and Research Unit to meet domestic supply of traditional drugs and to propagate research and development activities.

Pharmaceutical and Research unit; its nature and role.

The small cottage factory was established in 1982. The present large and well-furnished factory was built in 1998. Now, it meets the European GMP standards. The Unit formulates about 125 different traditional drugs and the annual yield has crossed 7 tonnes last year.

The standardization of raw material is in the process. More than 200 medicinal plants collected within Bhutan and around 20 herbs imported from India that are commonly prescribed in the traditional herbal formulations is subjected to astringent standard quality testing process. Anti-fungal and anti-micotic properties of about 25 Bhutanese medicinal plants were determined. *Rhodondron anthoogon of Ericaceae family* is one of them. Many medicinal plants that are endemic to Bhutan (*like Codonopsis bhutanica)* needs research, standardization and proper utilization schemes. The enthno-botanical and ethno-medical studies are yet to gain momentum.

Delphenium brononiánum (de-toxicant) and *Pleurospermum amabile* (antidote) which are both used as the substitutes for Musk in traditional formulations, gives bivalent scope for research. Firstly, to study if the substitute really and genuinely serves the therapeutic effect as that of musk *(used almost in 35% Formulations)* and secondly to find another substitute with the alike therapeutic properties for those two endangered plants.

Constraints

- Manpower shortage. The unit has no trained, experienced, skilled and qualified personnel to lead exhaustive research and development activities.
- The complexity of multi-ingredient herbal medicines hampers proper investigation and research.
- Shortage of rare and endangered raw materials.

Government prohibits procurements of the raw materials listed under the Forest Act as 'rare and endangered species of plants and animals'. Thus, Tug-of-war between the Conservation issues and the GMP ethics (safety, efficacy, and quality) has cropped up as such.

Solutions

- The priority is given to Human Resource Development in this field. We also recruit expertise and long term consultants from time to time.
- Cultivation and survey of medicinal plants in collaboration with the Agronomy section of Ministry of Agriculture is initiated. Some medicinal plants, including the rare species like *Delphinium brunonianum,* which grow above 5000 metres can be now successfully grown at 4300 metres in their private herbal gardens.
- We look forward for collaborative research on traditional herbal drugs.

Conclusion

Lastly, I would say that the use of the medicinal plants /herbs is deeply rooted in Bhutan's rich traditions and cultures. However, it demands systematic approach with good research, expertise of research, collaborative research and technical interventions. The researcher in the unit needs training, outlook and exposures. Therefore, I look forward for such kind of training courses and other related workshop that would take place in different countries in near future.

COUNTRY REPORT

COUNTRY: Cambodia

NAME: Mr. Sun Kaing Cheng

COUNTRY REPORT

By **Dr CHENG Sun Kaing**
National Centre for Traditional Medicine
Ministry of Health , **Cambodia**

Geography :

Covering an area of 181,035 square kilometers Cambodia is about half the size of Germany. In the West the country is bordered by Thailand ,in the North by Laos,and in the East by Vietnam .

Once a French colony is the least known Indochinese country. Cambodia has a distinct geographical personaly: it is a wide basin surrounded by highlands. In this basin the farmer has created a simple life-an original civilization and philosophy of midness. After many years of war, people rediscovered the meaning of "PEACE" . They started to rebuild and reconstruct in gems , fish,and has a big potential in tourism .

By far the most important river of Cambodia is the Mekong,which passes through the country for about 500 kilometers in a northsouth direction. The Mekong is passable for its delta in Vietnam until Phnom Penh.

Southeast Asia's largest lake,Tonle Sap,is in Cambodia and is connected to the Mekong by a short river,also called Tonle Sap. For most of the time this river flows from lake Tonle Sap into the Mekong. However,during the Southeast Asian rainy season from June to October when the Mekong drains large areas of Southeast Asia,the Tonle Sap river flows from the Mekong to the lake Tonle Sap thus causing enormous floods in the area of surrounding the lake. During this time, lake Tonle Sap con swell to more than twice of its regular size.

Central Cambodia is a fertile plain. Mountain ranges in the shape of a semicircle form of a natural boundary with Thailand. In the West are the Cardamom Mountains(designated after the spice of the same name),in the Southwest the Elephant Mountains and in the North the Dangrek Mountain Range. The highest mountain in Cambodia is Phnom Aural in the Cardamom range,at a height of 1,813 meters.

To date these mountain ranges are comparatively densely covered with forest and only sparsely populated. All three are still operating areas of the Khmer Rouge guerrillas.

-**Total Area:** 69,900 sq.miles
-**Population (1989 Estimate)** : 8,055,000
-**Capital** : Phnom Penh
-**Largest Cities** : Phnom Penh, Battambang, Kompong Som, Siem Reap, Kompong Cham, Kompong Thom
-**Largest Lake** : Tonle Sap
-**Major Waterway** ; Mekong River
-**Mountain Ranges** : Cardamom, Dangrek
-**Highest Point** : Phnom Aural, 5,948 feet
-**Land Usage** : About three-fourths tropical forest; roughly one-fifth arable land. Bulk of remaining land is composed of sandy and infertile soil.
-**Wildlife** : Animals found in Cambodia include monkeys, water buffalo, tigers, elephants, leopards, and crocodiles.

Government :

Type: Theoretically,Cambodia is a Constitutional Monarchy ;
Government is headed by democratically elected Prime Minister. The National Assembly is composed of 120 representatives. The voting age is 18.

Economy :

Primary Occupation : Agriculture
Chief Agricultural Products : Rice,rubber,cassava,sweet potatoes,corn,beans,tobacco.
Chief Manufacturred Products : Cement,rubber,cigarettes,wood products
Chief mined Products : Salt,rubies
Monetary Unit : Riel

Culture :

☛ Ethnicity : Khmer(approx.90%); Chinese(approx.5%); Vietnamese(approx.5%);

small minorities of hill tribes,Chams.Burmese,and Thai .

☛Religions : Theraveda Buddhism(95%);Islam; animism; atheism
The majority people of Cambodia are followers of Thervada and Hinayana school of Buddhism which was introduced to Cambodia between the 13th and 14th centuries , and it was designed as the state religion until 1975.

☛Languages : Khmer(95%); some French, Vietnamese, Chinese,and English

☛Literacy : approx.50%

☛Currency : The unit is the RIRLS. Riel denominations are 100, 200, 500 and 1000. Exchange rates are subject to fluctuation. US$ are generally accepted thoughout the country. In Phnom Penh , some credit cards can be used and limited services and purchasing and cashing traveler checks.

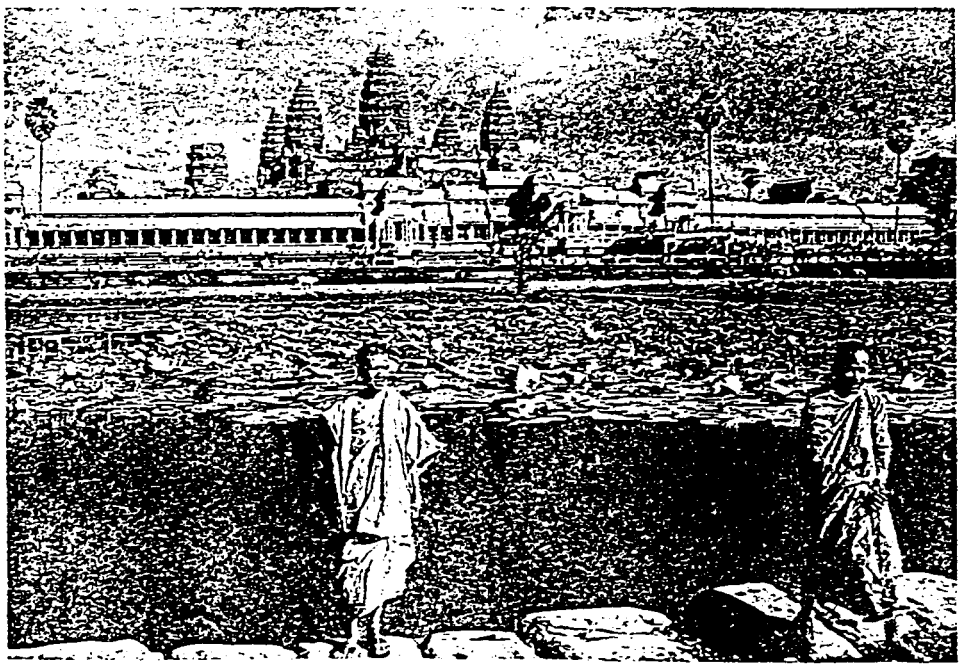
☛Food : Cambodian food is nearly related to the cuisines of neighboring Thailand and Laos,and to a lesser extent,Vietnam,but there are some distinct local dishes. In the growing number of restaurants in Phnom Penh and Siemreap , you will find excellent Chinese and Vietnamese dishes but it is the local dishes which are often the best prepared and most interesting. Rice is the principal staple and Battambang region is the country's rice bowl. Most Cambodian dishes are cooked in a wok known locally as Chnang Khteah.

☛Climate : Cambodia lies in a tropical zone between 10 and 14 degree of latitude north the equator. The temperature is fairly uniformed throughout the year , and average 25 degree centigrade(77degree fahrenheit). The relative humidity is higher at night and usually in excess of 90 percent , the average humidity during the day time is 80 percent. There are two seasons: rainy season and dry season. The climate is warm and humid(temperature is between 20°C-30°C .

How to reach Cambodia & Angkor Wat :

There are direct flights to the capital of Phnom Penh from Bangkok, Hanoi, Hochiminh City, Hong Kong,Kuahlupur,Singapore and Vientiane.From Phnom Penh,one can take direct flights to Siemreap, Angkor,which are now operates on daily basis.

Because of its vast architectures and plenty of myths and information,visiting of the great Angkor Wat a knowledgable and well-trained guide is highly recommended and fun. You can enjoy travell to Angkor Wat .



BRIEF HISTORY OF CAMBODIA TRADITIONAL MEDICINE:

The use of the traditional medicine has a particularly rich tradition among our people.

The traditional medical system in Cambodia made a great contribution to maintain Cambodian health.

Similary to other Asian countries, the history of the Khmer traditional medicine is closely linked to the history of its religion.

In Angkor era especially under the reign of the Emperor Jayavarman VII-12thcentury,Khmer Traditional Medicine was very developed. Hospital Health facilities network were everywhere throughout the country.

Under French rule-in the year of 50,modern medicines were taken part in the treatments,however,only the rich people could access it and traditional medicines had been used all over the country.

At Pol Pot time,either most of the remaining Bali books,the valuable documents,the experience of the most gifted intellectuals and monks,or good traditional healers had been destroyed or lost. During the same time,however,it seems traditional medicine underwent a period of renaissance,as,in most parts of the country,it represented the only type of medical treatment available at any level of society.

After 1979,the government has officially integrated traditional medicine into the national health system and has involved a policy of support that has promoted,at different levels but in an uncoordinated manner,a disparate number of activities,most of which are still going on at present.

A network of Traditional Medicine system was set up :

-In 1982,the Centre for "Research on Traditional Medicine and Pharmacy" was opened at central level and working with aid from:UNICEF,NOVIB,CIDSE,Fondation Danielle Mitterrand (FRACE-LIBERTES), and actually ,WHO.

-One TRM hospital in Phnom Penh using both traditional healers and medical doctors.

-In each district hospital and commune dispensary,traditional practitioners were accepted and a medicinal plants garden of most necessaries for the treatment.

-The Phnom Penh Municipal Health service had assembled and trained basic medical science to 230 declared Healers.

-At the borders,in camps, NGO's had trained some.

I-CURRENT STATUS OF TRADITIONAL MEDICINE IN CAMBODIA :

Today the network indicated as above has deteriorated,Traditional Medicine hospital is no longer worked ,it might be caused by:

-the Khmer TRM had lost its knowledge backing,
-traditional remedies are newly formulated,
-diffculties in collaboration between traditional healers and medical doctors,
-benefits problems,
-medical doctors do not wish to deviate from their real professional activities,
-lack of budget and resource persons.

❒ **IN PUBLIC SECTOR :** The National Centre for Traditional Medicine,Ministry of Health

National Centre for Traditional Medicine,ex-called Centre for Research on Traditional Medicine and Pharmacy ,had been started from zero with an inappropriate building ,resource persons missing and lacking of means. Because of the donation of different NGOs the Centre could have been run since. Now ,there are 6 pharmacists,1 assistant pharmacist,1traditional healer and other 30 staffs.

● **Activities** :

-Organized a survey of medicinal plants in 6 provinces. 514 plants having therapeutic value had been collected and listed.

-Developed booklet of 40 medicinal plants for common diseases, 3 volumes of medicinal plants (rhoneo-publishing), 2 volumes of illustrated brochures on "Medicinal Plants in Cambodia" and Your Medicines in Your Garden(a booklet contains 11 medicinal plants commonly used for Primary Care) funded by WHO.

-Developed a list of 16 selected diseases that can be treated by medicinal plants for using in Primary Health Care systems, a list of 47 other selected medicinal plants having industrial and economical interest. a list of 11 medicinal plants having toxic effect, and 12 toxic and venenous plants.

-Made medicinal plants into pharmaceutical forms, through clinical trials, the quality and efficacy of the products had been ensured. There are medicines for diarrhea, dysentry, toothache, cough, constipation, ear pain, and oitment for treating shingles and skin diseases.

-Extracted Berberin from Coscinium usitatum Pierre and made it into pharmaceutical forms : pomade, tablets, tincture for external use.

-Organized the first National Workshorp on Traditional Medicine and Natural Products , supported by WHO , on October 1997 held in Phnom Penh Cambodia.

-Medflore Database: 160 medicinal plants have been incorporated.

● **Equipments** :

At the disposal of making pharmaceutical products, National Centre for Traditional Medicine has possessed equipment for division, grinding, extracting, tabletting, and caoting. But the Laboratory for analysis of components existing in raw materials and for the Quality Controls were failed. The qualified personal working in this branch seem rare and need to be trained more.

● **Tasks** :

1 -To collect and record knowledge and information on Traditional Medicine;
2 -To select traditional remedies which could be used in support of Primary Health Care, and to select medicinal plants which may attract commercial or industrial interest;
3 -To identify diseases or disorders which could be treated by traditional medicine;
4 -To promote the appropriate use of traditional medicine in communities;
5 -To undertake study on conservation and reproduction of medicinal plants species;
6 -Manufacture of herbal medicine products;
7 -To provide the suggestion and recommendation to the Ministry of Health on issues related to Traditional Medicine ;
8 -To be National Coordinator for National Network on Traditional Medicine;
9 -To establish the necessary methods and technologies for the quality control to ensure the safety and efficacy of herbal medicines and to develop traditional remedies.
10-To develop monographs of 10-20 selected plants;
11-To create a small traditional clinic;
12-To continue Medflore Database.

❒ **TRADITIONAL MEDICINE IN USE** :

Herbal medicines had been used for thousands of years. The practice continues today becausee of its biomedical benefits and place in cultural beliefs everywhere throughout the country. Cambodia has the potential to make the use of national raw materials,more than 500 medicinal plants are valuable.

Usually the patient use grinded or chopped parts of medicinal plants in decoction, liqueur or powder form. The extract of the plant can be used in single or in compound mixed with other ingredients for external use or make it in tablets or pills form for oral use.

Thinking about health and the hope of living , and the rapid spread of HIV in Cambodia, we hope herbal medicines will contribute to the health care by holding up health condition and so extend the lifetime.

II-GOVERNMENT POLICY :

1- Organize research based on fundamental sciences and sciences to be applied on Traditional Medicine.
2- Research on diseases which are able to be treated effectively with Traditional Medicine.
3- An effort will be made to establish methodologies and technologies to develop traditional remedies.
4- Enhance the quality of traditional medicine to be appropriate to the science criteria.
5- Provide training to the professional health workers on Traditional Medicine and Pharmacy.
6- Promote the use of Traditional Medicine in Primary Health Care.
7- Increase the importance of Canbodian Traditional Medicine and encourage traditional practice as a complementary to modern medicine.

III-REGULATORY SITUATION OF TRADITIONAL MEDICINE :

There are :

1- Decision of Minister of Health on conditions of the opening, closing and changing traditional medicine store issued on 6 October 1998.
2- Sub decree about: production, importation, exportation, and traditional medicine trading for Health issued on 28 April 1998 by Royal Government.
3- Procedure for Regulation of Traditional Medicine locally produced and imported products issued by Department of Drugs-Foods-Medical Materials and Cosmetics/ MoH.

IMPLEMENTATION :

1- To promote the assurance of quality, safety and efficacy of traditional remedies using the updated technology.
2- To control private sectors regarding Traditional Medicine. To differentiate and separate activities of manufacturer, whole seller, retailer and healer.
3- To register traditional products.
4- To strengthen Nation Centre for Traditional Medicine to be able :
   -to carry out an inventory and survey of medicinal plants;
   -to find out what diseases can be treated with efficacy by traditional medicine;
   -to propose official list of traditional remedies to be used in Primary Health Care.
5- To collect, collate and catalogue various documents and specimens currently scattered aroud the country.
6- To train staffs for the identification of medicinal plants :
   -physical and chemical essays,
   -documentation of information,
   -different categories of specialties.
7- To provide the basic laboratory equipment to the National Centre for Traditional Medicine.
8- To prepare National Traditional Pharmacopoeia.

THANK YOU

NATIONAL CENTRE FOR TRADITIONAL MEDICINE

MINISTRY OF HEALTH , CAMBODIA

LIST OF MEDICINAL AND AROMATIC PLANTS

No	KHMER NAME	LATIN NAME	FAMILY NAME	CHEMICAL COMPOSITION	THERAPEUTIC USES
1	ក្រូចឆ្មារស្បើ	Citrus aurantium Lin.	Rutaceae	Cineol, Linalol	coughs, digestive
2	ក្រូចឆ្មារ	Citrus medica L.	Rutaceae	terpene, limonene, α-pinene	coughs, digestive, antispasmodic
3	ក្រូចពោធិសាត់	Citrus sinensis	Rutaceae	essential oils	coughs, digestive
4	ក្រវ៉ាញ	Amomum Krevanh Pierre	Zingiberaceae	α-pinene, α-terpineol, borneol	carminative,expectorant
5	ក្រូចសើច	Citrus hystrix DC.	Rutaceae	essential oils	Influenza
6	ក្រសាំង	Feroniella lucida	Rutaceae	essential oils	Influenza, digestive
7	ក្រូចថ្លុង	Citrus grandis Osbeck	Rutaceae	Citral	coughs, digestive
8	ក្រូចឃ្មិច	Citrus nobilis Swigle	Rutaceae	d-limonene, citral	coughs, digestive
9	ក្រូចព្រៃ	Atalantia monophylla	Rutaceae	essential oils	coughs, digestive
10	ការ៉ាយ	Allium odorum	Liliaceae	Odorine	coughs, digestive , carminative
11	ខ្ទាញ់ផ្កាលឿង	Spilanthes acmella	Asteraceae	stigmasterol , spiranthol	Analgegic, febrifuge
12	ខ្ទឹមបារាំង	Allium cepa L.	Liliaceae	d-menthol,d-limonene	Asthenia, fatigue

13	ខាន់ក្ពួ	Eugenia caryophyllus	Myrtaceae	eugenol , kaempferol	Local anesthesic,
14	ខ្ទឹម ស	Allium sativum L.	Liliaceae	alicine	Antivenimous, hypertension
15	ខ្ញី	Zingiber officinale	Zingiberaceae	zingerol,zingerone, shogaol	hypocholesterolemiant, antirhumatismal, tonic
16	ចេកទុំ ផ្កា	Artabotrys odoratissimus	Annonaceae	essential oils	diuretic,carminative
17	ចំប៉ា	Michelia champaca	Magnoliaceae	essential oils	carmonative,febrifuge
18	ជីត្រចៀកជ្រូក	Coleus aromaticus	Labiateae	coleine , carvacrol	coughs
19	ជីក្រសាំងទុំហំ	Polygonum odoratum	Polygonaceae	essential oils	alimentary toxicosis
20	ជីឃិនឆាយ	Petroselium sativum	Apiaceae	apiol, myristine	headaches
21	ជីគ័ហោ	Mentha arvensis	Lamiaceae	menthol,menthone	friction, digestive
22	ជីឆាវង	Ocimum basilicum	Lamiaceae	eugenol,geraniol, linalol ,eucalyptol	antispasmodic, carminative, digestive
23	ជីរណោ-ចាវ៉ាង	Eryngium foetidum	Apiaceae	essential oils	febrifuge, digestive
24	ជីវ៉ាន់ស៊ុយ	Coriandrum sativum	Apiaceae	linalol, d-pinene	digestive
25	ជីភ្លូ	Piper lolot G.DC.	Piperaceae	geraniol, borneol	carminative,diarrhoea
26	ដដ្ដួ	Piper cubeba	Piperaceae	essential oils, piperine,	migraine,rheumatism,boils
27	ទេពភីរូ	Cinnamomum cambodianum	Lauraceae		carminative, digestive
28	ពាយម៉ាត	Blumea balsamifera	Asteraceae	essential oils	luxation, febrifuge

29	ដើមប្រេងខ្យល់	Eucalyptus globulus	Myrtaceae	cineol,eucalyptol,cajeputol	febrifuge,antiseptic
30	ផ្កាស្បែរឿង	Tagetes erecta	Asteraceae		coughs,desintoxication
31	ផ្កាម្លិះ	Jasminum sambac	Oleaceae		antispasmodic
32	ផ្កាសារិកាតែវ	Murraya exotica	Rutaceae	essential oils,exoticin	diarrhoea, febrifuge
33	ផ្កាច័ន្ទ-ប៉ូចកាក់	Illicium verum	Magnoliaceae	anethol	carminative,antispasmodic
34	ម្រះព្រៅក្រហម	Ocimum sanctum	Lamiaceae	eugenol	antispasmodic,carminative
35	ម្រះព្រៅ ស	Ocimum gratissimum	Lamiaceae	gratissimine,eugenol	influenza, sudorific
36	ម្ទេ	Piper betle	Piperaceae	cineol,eugenol,cathecol	carminative, coughs
37	ម្រេចខ្មៅ	Piper nigrum	Piperaceae	essential oils,piperine	digestive, carminative
38	រមៀត	Curcuma longa	Zingiberaceae	curcumene,curcumine	haemostasis,gastritis, pneumonia, flatulence
39	ស្នាច់ដោម	Eugenia zeylanica	Myrtaceae	cajeputol , eucalyptol	antalgic,healing,sprain
40	ស្នាច់ចន្ទ្រះ	Melaleuca leucadendron	Myrtaceae	cajeputol, eucalyptol	coughs, febrifuge,sprain
41	ស្មៅក្រវាញជ្រូក	Cyperus rotundus	Cyperaceae	cyperene, cyperol	emmenagogue , colic , diuretic
42	ស្លឹកត្រៃ	cymbopogon nardus	Poaceae	citral,citronellol, geraniol,citronellal	febrifuge, carminative

Phnom-Penh, 24th July 2000

COUNTRY REPORT

COUNTRY: Ethiopia

NAME: Ms. Abeda B. Kassaye

Research On Medicinal and Aromatic Plants In Ethiopia

(Status Report)

Essential Oils Research Center

P.O.Box 3395

Addis Ababa, Ethiopia

August, 2000

1. BACKGROUND

Ethiopia is a country of wide range of climatic conditions. The country has areas ranging from below sea level up to over 4260m. a. s. l. The wide range of climatic variation enables the country to possess an enormous diversity in plant genetic resources. Variation in altitude makes it also possible to introduce plants not indigenous to the region.

As in most developing countries, Ethiopian people are culturally bind to the use of traditional medicines. Among several traditional health practices in Ethiopia, the use of herbs is the major one. Traditional healers keep the knowledge of the therapeutic qualities of the country's flora as a secret and entrust it only to their successors in the job (usually old son) as a heritage.

The majority of the rural populations of developing countries have difficulty affording Western Medical health care. Typically, more than 80% of health Budgets in developing countries are directed to services that reach approximately 20% of the population. Of this, 30% of the total health budget is spent on the national pharmaceutical bill (Bannerman et al., 1983). Like wise, it has been reported that more than 85% of the Ethiopian rural population have no access to modern medicine. Those people relay on traditional medicine, which is based on curative plants (P.C.M.Jansen, 1981). Therefore, the call for the promotion of use of Medicinal plants has economic and culturally significance.

The region in which Ethiopia is located (along the equator) is rich in flora (medicinal and essential oil bearing plants) possessing an enormous diversity in plant genetic resources. The country's endowment of various favorable environmental conditions gives also possibility to introduce and cultivate exogenous plants.

Some of the common Traditional Medicinal Plants of the Country

Scientific name	Vernacular name	Reputed Usage
Adhatoda schimperiana	Sensel	anti malaria
Ajuga integrifolia	Armagusa	anti dysentery
Aloe spp.	Eret, seteret	laxative, eye and ear disease
Brucea antidysenterica	Woogions	anti dysentery
Catha edulis	chat	stimulant
Chenopodium sp.	Damakesse	fever, eye disease
Croton macrostachyus	Bisana	purgative, anthelminitic
Cymbopogon martinnii	Tej-sar	heart, chest & stomach complaints
Datura spp.	Atefaris	stimulant, pain killing massage
Echinops sp.	Kebericho	common cold, wound dressing
Embelia schmperi	Enkoko	against worms
Eucalyptus globulus	Nech bahir zaf	common cold
Euphorbia abyssinica	Kulqual	disinfectant
Hagenia abyssinica	Kosso	against flat worm
Lepidium sativum	Feto	stomach cramp
Nigella sativa L.	Tikur azmud	headache
Olea africana Miller	Woira	anthelmintic
Phytolacca dodecandra	Endod	bilaharzia
Ricinus communis L.	Gullo	haemorrhoids
Rumex Abyssinicus	Mekmeko	gonorrhoea
Rumex nervosus Vahl	Embacho	haemostatic, skin disease
Spilanthes mauritiana	Ye' midir berbere	analgesic
Stephania abyssinica	Aregiat	anti dysentery
Tavernnera abyssinica	Dingetegna	muscle contraction, headache
Thymus schimperi	Tossign	anti hypertensive

In spite of the existence of such varied flora in Ethiopia, processing of the derived chemicals or isolation of active principles is not done due to the lack of suitable scientific and technological know how and infrastructure necessary for the development of appropriate technology and its transfer to industry.

Concerning the pharmaceutical production in Ethiopia, there are four Ethiopian Pharmaceutical Enterprises (EPHARM, Addis Pharmaceutical Factory Plc., East Africa Pharmaceuticals Plc. and BIOSOL Pharmaceuticals Plc.). All formulate medicines from imported synthetic drugs.

The limited availability of foreign currency and the high prices of pharmaceuticals aggravated by fluctuating exchange rates have made and will continue to make these synthetic origin medicines beyond the reach of the increasing number of people in Ethiopia.

The country having a large rural population it is very essential to develop agro-based industry such as the production of medicinal and aromatic plants and their derivatives. And this blends well with the agro-based development plan of the country.

Research activities on Aromatic and medicinal plants offer great opportunities to the development of pharmaceutical industry that formulate extracts from the countries abundant and symbiotically human related medicinal plants. Emphasis should be given to the cultivation of plants in order to conserve them and have sustainable supply.

2. RESEARCH AND DEVELOPMENT ACTIVITIES

In Ethiopia there are few organizations engaged in Research and Development activities of aromatic and medicinal plants. The activities in these organizations are fragmented and with minimum collaboration linkage. Most of the research works are confined to academic areas like universities, which conduct academic research concentrating on extraction, isolation of components and structure elucidation in the laboratory for awarding degrees and publication of research findings.

The major research institution engaged in medicinal plants R&D is Department of Drug Research (DDR) of the National Health and Nutrition Institute.

2.1 Department of Drug Research (DDR) of the National Health and Nutrition Institute

The department is engaged in the study of modern medicines and medicinal plants used traditionally. The research work covers activities from identification of medicinal plants to drug development (formulation). The department conducts its research works in collaborations with volunteer traditional healers and various relevant departments of the Black Lion medical faculty of Addis Ababa University.

Progress at DDR

- The department has set up a computerized database that housed information on many traditional medicinal plant species.
- The department has phythochemistry, pharmacology, microbiology and formulation laboratories.
- A herbarium with a collection of 92 families and more than 600 species of traditionally used medicinal plants is established at the department.
- Biological and pharmacological screening is being done on selected traditional medicinal plants. The selection is based on health problem of the country, international consideration of the specimens and production simplicity.
- The preliminary biological and pharmacological screening on some of the medicinal plants carried by the department revealed that some plants have very promising therapeutic potential. They are under intensive trials.
- Formulation has been done for some plant extracts for demonstration purpose. This includes :-
    - Syrup from Senna, Ginger and Garlic
    - Ointment preparation from Trachyspermum ammi
    - Herbal tea from senna
- Conservation work is being done for medicinal plants that are becoming extinct.

2.2 Essential Oils Research Center

The Essential Oils Research Center (EORC) is a governmental institution engaged in R&D and pilot production activities on essential oils and other plant based chemicals. The center evolved in 1992 from the Essential Oils R&D unit under the National Chemical Corporation (NCC). Now it is organized under the guidance of the Ministry of Industry with moderate government allocated budget.

EORC has its head office and main laboratory in Addis Ababa and an agricultural research site covering around 80 ha. of irrigable land, a mini laboratory and a French type pilot plant distiller of green herb at Wondo Genet which is located at about 270 km south of Addis Ababa.

More than 200 species of essential oil bearing plants, insecticidal plants, medicinal plants, vegetable tannin, oil seeds, etc. are found in the center. Among these four types of essential oils from the plants Cymbopogon martinnii, Cymbopogon citratus, Eucalyptus citriodora and Eucalyptus globulus are being produced in pilot scale for demonstrative purpose. The oils produced are sold to local users. Methods of product maximization are also studied.

The moderately equipped laboratory of the research center is built and assisted by foreign donors such as the Swedish Agency for Research Cooperation with Developing Countries (SAREC/SIDA).

2.2.1 Objectives

- To conduct agronomic, biological & chemical studies on plants of industrial use like Essential oil bearing plants, Insecticidal, Medicinal plants, Non-edible oil seeds, etc.
- To adopt & develop technological packages for the processing of plant derived chemicals.
- To carry out demonstration & small-scale production of plant derived chemicals.

- To promote, stimulate, organize and direct multidisciplinary research on aromatic & traditional medicinal plants in the country.
- To give consultancy, laboratory, information & library services.
- To contribute significantly to the development of the agro-industry, in product diversification and in saving and earning of foreign currency.

2.2.2 Research Center's Activities

- Collection and selection of appropriate plants from different areas. This could be locally or from abroad.
- Agronomic studies are being carried out on some of the collected plant species. The trials include fertilizer type & rate, harvesting time, frequency and other relevant parameters, which are believed to influence quality and yield of oil.
- Identification of various regions of the country where essential oil bearing plants could be cultivated has been accomplished. Further studies and verifications will continue.
- Developing technological package for the studied plants.
- Chemical analysis and quality control is being carried out for some essential oil bearing plants.
- Distributing product samples to local soap and cosmetic factories and end users to substitute imported essential oils.
- The center works in collaboration with different institutions. Some of these are :-
  - Institute of Water Technology in a research on natural coagulants for water purification.
  - Ministry of Coffee & Tea on the promotion of matricaria recutita (Chamomile) as a herbal tea.
  - Chemistry department of Addis Ababa University for the instrumental analysis (GC/MS, NMR etc) of most of the research work.

2.3 Institute Of Biodiversity Conservation and Research (IBCR)

IBCR is a governmental institution mandated to collect and conserve germplasm of plants, animals and microorganisms. Recently, the institute has proposed and developed a project on the conservation and sustainable use of medicinal plants in Ethiopia. The project is financial supported by World Bank and Global Environmental facility (GEF).

Fifteen stakeholders are involved in the project. These include traditional healers Associations (THA), National Health and Nutrition Institute, Ethiopian Agricultural Research Organization (EARO), EORC, various departments of Addis Ababa University, regional government, etc.

Objectives of the Project

- To establish medicinal plants field gene bank.
- To develop intellectual property right policy and guideline.
- To survey socio-economic benefit of medicinal plants to human being and livestock at national level.
- To conduct research on the propagation and cultivation of medicinal plants.
- To conduct formulation study. This includes extraction, standardization, and safety and efficacy study and dosage formulation.
- To conserve medicinal plants *IN-SITU* at Bale Mountains Natural Park, which is, considered as one of the 200 Eco regions.
- To cultivate medicinal plants in buffer areas for sustainable harvest.
- To create awareness by giving training to park administrators, traditional healers and the public at large.

2.4 Other Institutes Dealing with Medicinal and Aromatic Plants

Chemistry Department of Addis Ababa University

It is working on various phytochemicals through the special-Natural Products Research Unit that deals with isolation and characterization of individual substances from plant materials. The research activities of this unit are not only confined to medicinal plants but also with plants having great potential to the food, perfumery and other chemical industries. Chemical constituent analysis is done for these plants of diverse applications.

Biology Department of Addis Ababa University

It is the pioneer in the medicinal plant research especially in collection and identification of plant species. The National Herbarium at the department has a collection of about 50000 specimens among which about 500 are medicinal plant species.

School of Pharmacy of Addis Ababa University

It is also a pioneer in medicinal plant research. Through its various departments it has carried out a number of biological and pharmacological studies on medicinal plants.

Institute of Pathobiology of Addis Ababa University

It has done a lot and still is working on the Ethiopian high land plant "Phytolocca dodecandra" locally known as "ENDOD" which is used locally as effective soap with strong bleaching action. "ENDOD" was found to possess a pronounced moluscicidal property against snails transmitting abd a rapidly spreading parasitic disease known as schistosomiasis. An Ethiopian Biomedical scientist Aklilu Lemma discovered this property of "ENDOD".

Among the various plant species "ENDOD" has reached the stage of commercialization.

3. Industrial Production

Aside from the pilot scale production of EOs, which amounts to about 2-4 tones/year, there is no production of essential oils in the country. Recently, investors are coming to the sector. An

Italian couple already started cultivating medicinal and aromatic plants farm named Bishoftu Medicinal and Aromatic Plants (BIMAAP).

There are two spice extraction factories engaged in the production of oleoresin of paprika & ginger, the Ethiopian Spice Extraction Enterprise and KASSK Spice Extraction Company which have an annual installed capacity of 155 and 120 tons of production per year respectively.

4. Local and Export Market Situation

One to two tons of essential oil is sold locally in each year. Export of oleoresin from capsicum and ginger is well established. Quite a substantial amount of frankincense (aromatic gum) is also exported, although very insignificant export of Eos has been started.

5. Constraints to the Development of the Sector

The production of plant derived chemicals, being relatively new to the country, there are a lot of constraints to develop this sector. These include: -

- The awareness in the field is far from satisfactory.
- Agricultural practices which suits the local conditions are not yet developed.
- Trained manpower and know how in the field are very limited.
- Laboratory equipment, consumables etc. needed for quality specifications are not available.
- Sufficient attention is not given to the sector. The country being food deficient, priority is given to food crops followed by traditional cash crops.

6. Technical Assistance Requirement

Research and Development in the field of Eos and medicinal plants in general can be said that it is at its infant stage. Very limited experts are working on aromatic and medicinal plants. Developing aromatic and medicinal plant production and processing in the country necessitate

technical assistance in training, technology transfer, market information, laboratory equipment and pilot scale units.

Technical cooperation among developing countries in the region could solve some of the problems. This could be through:

- Exchange of authenticated plant materials
- R & D know-how exchange
- Package development for specific crops
- Adoption of technology
- Collaborative works enabling sharing instruments needed for the analysis

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COUNTRY REPORT

COUNTRY: Indonesia

NAME: Ms. Berna Elya

Country Report and Faculty of Science and Mathematics

-Indonesia is a big country which is rich in precious medicinal plants. These people used to utilize herbal and medicinal plants for the prevention and treatment of disease. This traditional medicine knowledge has been handed down from generation to generation up to the present day.

-Due to economic crises, the Government of Indonesia has tried to develop alternative medicines through utilization of medicinal plants. The intention of the Government is how to provide medicines for the Indonesian people which the prices are affordable by the people. The government has seriously fostered several researches to explore the natural resources which is available in Indonesia.

-The researches on medicinal plants have been conducted by several institutions, such as:

-Research and Development of Health Department

-The Indonesian Research Institute (LIPI)

-The Agency for Development and Application of Technology

-Universities

-Industries.

Medicinal plants:

- The National Working Group on Indonesian Medicinal Plants (POKJANAS -TOI)
- Association of National Natural Medicine (PERHIBBA-NAS)

This year, several congress and national seminars have been conducted by those institutions.

1. The National Seminar XVII Indonesian Medicinal Plants, March 28-31, 2000 In Bandung. The National Seminar conducted by the National Working Group on Indonesian Medical Plants (POKJANAS TOI) in cooperation with the Indonesian Research Institute - Bandung branch. The topic of the seminar is "An Exploration, Conservation, Development and Utilization of Medical Plants 'Quisqualis indica (called 'Ceguk' in Indonesia) and '*Gastrochilus panduratum* Ridl (called 'Temu Kunci in Indonesia) Research came from Hasanudin University, Airlangga University, Gajahmada University, Bandung technology institute, Padjadjaran university, Andalas university. Every six months POKJANAS TOI arrange the seminar with two kinds plants.

2. The National and Sciencetific Congres of the Indonesian Pharmacyst Association, Jakarta, April 23-27, 2000. The researchers from Pharmacognosy and Phytochemistry group presented 17 papers, meanwhile from Pharmacology and Microbiology group presented 27 research papers on medicinal plants. The attendants came from pharmacysts in Indonesia.

3. One Day Seminar on The Utilization of Natural Product, Jakarta, June 28, 2000 and The Workshop on "Extraction, Standardization on Extract and Formula" conducted by Association of Natural Medicine-branch Jakarta (PERHIBBA-branch Jakarta) in cooperation with the Tujuhbelas Agustus University.

4. The National Seminar XVIII on Indonesian Medicinal plants will be conducted in Jakarta, September 6-7, 2000. The seminar will be organized by Department of Pharmacy, Faculty of Science and Mathematics, University of Indonesia in cooperation with the National Working Group on Indonesian Medical Plants in cooperation. The topics of seminar is 'An Exploration, Conservation, Development and Utilization of Medical Plants '*Anacardium occidentale* L. and *Curcuma heyneana* Val & V. Zijp'

5. The National Seminar XI on The Natural Product will be conducted in Surabaya, November 2000. The Seminar will be organized by Faculty of Pharmacy, University of Airlangga in cooperation with Association of National Natural Medicine (PERHIBBA-NASIONAL).

The Faculty of science and Mathematics, University of Indonesia is composed of six department, i.e, Department of Pharmacy, Department of Biology. Department of Chemistry, Department of Mathematics, Department of Geography, and Department of Physic Department of Pharmacy is compose of Devision of Phytochemistry and Pharmacoqnosy, Division of Formulas, Division of chemistry and division of Pharmacology.

As part of the Organization, the Devision of Phytochemistry and Pharmacognosy has been taking its prime responsibility in the research areas involving medicinal and aromatic plants. The research projects currently conducted in the devision can be categorized into two main areas :

1. Phytochemical studies. This research area includes the search for biologically active compound found in medicinal plants.
2. Standardization of herbal preparations.

ACKNOWLEDGEMENTS

I would like to thank ICS and UNIDO for giving me this opportunity to upgrade my knowledge and to develop contacts.

COUNTRY REPORT

COUNTRY: LAO P. D. R.

NAME: Ms. Monekham Sengsavang

Lao people's Democratic Republic
Peace Independence Democracy Unity Prosperity

Ministry of Public Health
Traditional Medicine Research Center

Country paper

Presented by

MONEKHAM SENGSAVANG

at

ICS-UNIDO training course on Research strategies on Medicinal and Aromatic plants, 14-18 August 2000, Bangkok, Thailand

Country paper

(a Training course on" Research Strategies on Medicinal and Aromatic plants")

Monekham Sengsavang

1.Introduction

The Lao PDR, a land-locked country has an area of 236,800 sq km of which 47% is covered by forests. Laos is one of the countries in Asia which is rich in precious medicinal plants .According to the survey of medicinal plants by TMRC (Traditional Medicine Research Center) in some provinces 2,365 species have been documented of which more than 500 species are used in various prescriptions. If we have appropriate measures to exploit all the latent potential of the medicinal plants and traditional medicine we can partially solve the problems of drug supply in remote areas. We are well aware that more than 85% of the population lives in rural areas. These people used to utilize herbal and medicinal plants for the prevention and treatment of diseases. This knowledge has been handed down from generation to generation up to the present day.

2.Status of Herbal and medicinal plant industry

Since the founding of the Lao PDR, a number of policies have been formulated by the government to promote the use of medicinal plants and traditional medicine. Nevertheless, there are few institutions dealing with research and development, production of herbal and medicinal plants in Loa PDR..

Looking at the public sector there are some commercial production facilities catering mostly for domestic market: they are

Firstly the Traditional Medicine Research Center(TMRC) : Since 1996 this Center started to plant Artemisia annua L. and extract artemisinine in its pilot plant this research was funded by the government of Vietnam.

Secondly the Pharmaceutical Development Center(PDC): This center was funded by the government of Japan for the production of conventional medicines and extraction of active principles from plants.

Thirdly the Pharmaceutical Factory No.2: Started to produce some medicine from plants in1994.

Fourthly the Pharmaceutical factory No.104: Most of the products from this factory are based on herbal and medicinal plants.

In the private sector: There are three home-based industries which produce traditional medicine for the people living in both urban and rural areas: they are

- Golden mouse Brand
- Naga (Serpent) Brand
- Tiger Brand

The newly established company, BIOIL, which is working on two projects: cultivation of aromatic plants and distillation of them, this company has a plan to produce essential oils.

Since Laos is a small country, at present there are few industries dealing with herbal and medicinal plants. In spite of the fact that demand of raw material from plants for pharmaceutical factories is fairly attractive, up to the present time there is no big business covering this field. Firstly, it may due to the lack of qualified personnel and appropriate materials. Secondly, the shortage of information

concerning research and development and the international market inhibits the expansion of our development in these areas. Finally, constraints of financial support from the government limits our ability to develop the work necessary for the supply and demand required by the people of our country.

In order to exploit all latent potential of our herbal and medicinal plants for the benefit of the people's health, technical assistance from international organizations and relevant institutions of friendly countries is needed for the following project of the TMRC such as:

- Rehabilitation of the production unit, including analysis equipment.
- Technology transfer.
- Short term consultant
- Short and long term training abroad.
- Exchange of information regarding research and development on herbal and
- medicinal plants.

Furthermore, I think that technical cooperation among South Asian countries is also important, because these countries are facing similar problems in their daily areas of concur. Therefore, our institute is ready to cooperate with other institutions for mutual Interest and benefit of all. The exchange of information can minimize duplication of efforts through joint planning of research and development project and conducting complementary project In the near future, we would like to carry out a survey of plant species having curative properties and their potential economic values, such as aromatic and medicinal plants. In order to accomplish this plan, assistance for domestic and international institutions vital because this work is difficult and takes

time. We should have appropriate means and qualified personnel to implement this plan. The outcome would provide basic data concerning the quantities of our medicinal plants. By this method the harvest of herbal and medicinal plants will not cause any problems for the environment and hence will not to nature.

Finally the Traditional Medicine Research Center , is grateful to the UNIDO, Research and Development institute , Government Pharmaceutical Organization of Bangkok for inviting us to participate in this program which would be beneficial to the future development of our cooperation and to the whole areas of traditional medicine.

COUNTRY REPORT

COUNTRY: Malaysia

NAME: Mr. Kok Seong Lim

RESEARCH AND DEVELOPMENT ON MEDICINAL PLANTS IN MALAYSIA

Current Problems

In Malaysia, the practice of traditional medicine is common among various ethnic groups i.e. Malay, Chinese and Indian. This traditional medicine knowledge has been passed on through many generations. Although traditional medicine is still commonly practiced and diverse species of medicinal plants have been used in its preparations, its quality and manufacturing procedures are still not well established. Currently, plant materials are mainly imported from foreign countries like Indonesia, China, Korea, with some collected locally from the wild. This often results in inconsistent supply and quality of raw material. Besides, this has also caused an outflow of large amounts of the country's cash reserve. One way of reducing this dependence is through thorough research on wild medicinal plants that have economic potential and are highly demanded by the local medicinal plant industry.

Research on Malaysian medicinal plants has been carried out for more than one decade, more than 90 percent of the research activities in local research institutes are, however, still based on discovery of bioactive component from plants. Most of the researches that have been carried out are 'basic' researches that allow the investigators to meet their academic interest or to pursue a degree. This is because the results of the researches can easily be presented in journals, seminars or conferences at international level, through which the investigators could gain reputation in their respective fields. Although these basic researches are essential, the results are not benefiting the community because they are not translated into development of products of commercial value, and only become thesis that lie on the bookracks in library. Hence, an organized research approach should be undertaken to ensure the linkage between basic research and research on product development.

On the botanical aspects of medicinal plants, until now Malaysia does not have a complete and well-documented Materia Medica as a standard and up-to-date reference. King & Gamble were the first two British botanists who made an attempt to describe and compile a comprehensive account for the flora of Peninsular Malaysia. However, it was Ridley who eventually managed to complete and publish the flora, in five volumes, between 1922 and 1925. In this flora, excluding those of ferns and fern-allies, a total of 6,766 species in 1,407 genera and 166 families were described. In 1926, Ridley enumerated the fern-flora of Malays Peninsular in which a total of 417 species in 86 genera and 16 families were described. Subsequent to Ridley's flora, a number of specialized botanical accounts that can be used for identifying plant species appeared. For Sabah and Sarawak, however, no such floras have ever been produced. Since extinction of species inevitably leads to a complete loss of genetic material, and thus also all its beneficial attributes including the medicinal properties, it is imperative that effort should be made to survey, identify, conserve and utilize plant genetic resources that are available in the Malaysian tropical rain forest.

In research and development on Malaysian medicinal plants, there is also a lack of communication between industries, scientists and government agencies. Inadequate coordination

among those who are involved explains why Malaysian community does not benefit from the results of the researches. At the moment, manufacturers in herbal industry does not understand the function of government agency e.g. National Pharmaceutical Control Bureau in registering herbal product, and governing bodies are not aware of the financial situation faced by local manufacturers. Cooperation among these bodies should be built in order to improve the herbal industry in Malaysia, and this can be achieved through meetings, conventions, seminars and workshops that involve all the representatives from respective fields. Other problems include lack of facility and funding and shortage of trained manpower.

There remains an urgent need for well-coordinated research programme involving botanists, phytochemists, pharmacologists, microbiologists and medical scientists, which can lead to the publication and dissemination of basic and applied scientific information, which is essential for the development and commercialization of traditional medicine.

Research Institutes in Malaysia

There are several research institutes and universities in Malaysia that are carrying out research on medicinal plants. Examples are Forest Research Institute Malaysia (FRIM), Malaysian Agriculture Research and Development Institute (MARDI), Putra University of Malaysia (UPM), University of Malaya (UM), University of Science Malaysia (USM) and National University of Malaysia (UKM).

Forest Research Institute Malaysia

Historical Background

Forestry research in Malaysia was formally organized in 1918. The Research Branch was then transferred to the present premise at Kepong in 1929. The Branch was named the Forest Research Institute (FRI), Kepong. In 1985, the Malaysian Forestry Research and Development Board Act was passed which allowed the Institute to change its status to that of a statutory body called Forest Research Institute Malaysia (FRIM). FRIM is now responsible to the Malaysian Forestry Research and Development Board (MFRDB).

The medicinal plant division was established in January 1995 under the mandate by the government that FRIM was identified to lead the national research activities in medicinal plants. In the bioprospecting programme, the medicinal plant division adopts a strategy that encompasses both a short term and long term plan which focus on the development of herbal product and identification of lead compounds.

Research and Development

FRIM draws up a five year Research Programme under each of the Malaysian Development Plan Period. The present Research Programme consists of 12 programmes that are the target areas in forestry for the Seventh Malaysian Plan (1996-2000). This Research programme has been discussed in depth by the researchers in FRIM and later endorsed by members of the Research Advisory Committee (RAC)

and subsequently approved by the Malaysian Forest Research and Development Board. The Research Programme associated with natural product discovery is aimed to:

a. develop modern processing technologies to convert phytochemicals into herbal products which are of high quality and safe for use by the general public
b. identify lead compounds in the development of new drug candidates for anti-infective, anti-inflammatory and antioxidant activities
c. provide standard procedures in sample preparation, extraction, chemical, chemical analysis and identification of marker compounds for medicinal plants used in the production of herbal medicine
d. gather comprehensive information on the propagation and cultivation of selected medicinal plants

Three main research activities in medicinal plant division of FRIM from 1996-2000 are:

a. Searching for new anti-inflammatory agents from plants

   Inflammatory related diseases such as arthritis, asthma and rheumatic fever are an important health problem. In this context, medicinal plants used in traditional medicine offer a fertile source of potential chemical compounds in the development of anti-inflammatory drugs. A range of Malaysian medicinal plants have been tested, via both in-vitro and in-vivo assays, to assess the biochemical pathway and mechanism of the anti-inflammatory action, as well as to ascertain the physiological action of the extracts on animals. Such assays included the PAF inhibition, heat-induced haemolysis, enzyme inhibition assays, the in-vitro TPA-induced mouse ear oedema and the carrageenan-induced rat paw oedema in vivo assays. Work conducted so far has uncovered encouraging results for several local plants which included Thottea grandiflora, Solanum torvum and Piper betle.

b. Identification of antioxidative component

   Plants, naturally high in antioxidant properties, supply the body with essential dietary components to supplement their natural defense systems. In Malaysia, ulam or raw vegetables, are consumed daily as salad. In FRIM, twenty different species of ulam such as kesom, kadok (Piper sarmentosum), pegaga (Centella asiatica), ulam raja (Cosmos caudatus) and selom were collected and tested for antioxidant properties using two different assays. The first assay is 'Autoxidation of Linoleic Acid' which shows inhibition on lipid peroxidation. The second one is 'X/XOD Superoxide Scavenging' assay which shows scavenging of active oxygen. The results obtained showed that eight types of ulam strongly inhibited lipid peroxidation (>96%) and seven types of ulam strongly scavenged active oxygen (>86%).

c. Investigation into anti-infective properties of plants

   Many local medicinal plants are used in treating common skin infections such as acne, pimple and ring-worms. They are therefore potential sources of phytochemicals or natural herbal extracts for use in the pharmaceutical and healthcare industries. They could be a safer alternative to the current use of synthetic material with a wide application in

products such as ointments, skin creams and liniments, hair and body shampoos or soap and facial cleanser. Target microorganisms include Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, P. cepacia, Candida albicans, C. intermedia, C. lipolytica, Trichophyton rubrum, T. mentagrophytes and Microsporum canis.

The upcoming Research Programme includes 9 programmes that are identified to be priority research area in forestry to be undertaken in the period of the Eighth Malaysian Plan (2001-2005). The ninth programme that involves medicinal plant division is concerned about natural products discovery. It is further divided into three subprogrammes:

a. Agronomics of medicinal plants and screening for bioactive compounds in plants

   The government emphasizes on the R & D of local medicinal plants obtained from the forests. There would be a concerted effort to concentrate on production of plants known for their effectiveness against certain "diseases" rather than diverting the limited human resource to research on the numerous areas. The research focus will be on plants effective against diabetes and hypertension, plants with potential for cancer/aids treatment, and plants with aphrodisiac properties.

b. Development of nutraceutical and herbal products

   In general there are few standardized protocols for production of nutraceutical and herbal products and the processes are handed down from one generation to another with incomplete documentation. For the production of products with consistent quality and effectiveness much work is needed to document, elucidate and improve the production process. This subprogramme will be focusing on improvement of processing technology for production, standardization of herbal plant materials, standardization of product formulation and development of standard protocol for quality control

c. Malaysia-MIT (USA) biotechnology partnership programme (MMBPP)

   The Malaysia-MIT Biotechnology Partnership Programme is a partnership venture in research and development with the main objective to develop advanced technologies that command the future of biotechnology. It is a five-year programme that is being implemented as two research sub-programmes:

   i. sub-programme 1: Natural Product Discovery

      Sub-programme 1, coordinated by the Forest Research Institute Malaysia, is directed towards natural product discovery from Malaysia indigenous medicinal plants, namely, Eurycoma longifolia (Tongkat Ali), and Centella asiatica (Pegaga). The research on Eurycoma longifolia focuses on: (i) in vitro propagation via somatic embryogenesis, (ii) quantitative measurement of the chemical or bioactive constituents, and (iii) development of standardized commercial formulations. Similarly for Centella asiatica, studies including identification and characterization of accessions, genetic fingerprinting, bioassays and bioreactor feasibility are aimed at the enhancement of bioactive metabolite production.

   ii. sub-programme 2: Oil Palm Biotechnology

Sub-programme 2, coordinated by the Malaysian Palm Oil Board (MPOB), employs new emerging technologies to improve oil palm (Elaeis guineensis) through tissue culture and metabolic engineering.

Conclusion

The medicinal plant division of FRIM is still in its infancy, the needs of integrated and multidisciplinary inputs are very critical for the success of the division research programme. Through the achievements of teamwork supplemented by strong technical skills at all levels, the division goals will be achieved.

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Forest Research Institute Malaysia 1999 Annual Report

Forest Research Institute Malaysia 1998 Annual Report

COUNTRY REPORT

COUNTRY: Nepal

NAME: Dr. Chiranjivi Regmi

COUNTRY PAPER ON STATUS OF MEDICINAL PLANTS IN NEPAL

C. Regmi
RONAST

Country Background

Nepal extend along the Himalayan range between the latitude of 26'22' N and 30'27' N and longitude 80. 04'E and 88° 12 E. The country has a length of about 885 km and average widh of 193km. It has an area of 147,181 sq. km and ranges from the Gangetic plain of Tarai (at about 60m) to Himalaya reaching over 8,800m in altitude.

With increasing altitude the vegetation changes from tropical in Terai to subtropial deciduous forest between 900m to 1200 m. in outer foothills and doon valleys. The lower temperate mixed forest occupies a zone from about 1500m to 2100m and upper temperate forest occurs at about 2400 m to 3200m and subalpine forests occur beween 3700m to 4600m. Above 4900m to the snow line there is discontinuous perennial herb.

Introduction:

Nepal is rich in floral diversity. It contains 5% of the floral sps of world. Nepal has a diversity of 6 phytogeographical provinces, 10 bioclimatic zones, 35 forest types and 75 vegetation types. The forest resource has outstanding significance in Nepal. It provides the economy to rural population with many products and serves important ecological function. It contains about 65000 spp of flowering plants including 280 spp of fern and its allies of which 250 spp are endemic to Nepal about 700 plant spp are known to have medicinal properties.

General Status and Trade

There is an estimation that 33 medicinal plants are collected and exported to India. The medicinal plants with annual collection exceeding 100 tons are, *Acacia concinna*, *Asparagus racemosus*. *Bergenia ciliata*, *Cinnamomum glaucescens*, *Lichens*, *Nardostachys grandiflora*, *Picr ohiza scrophulariiflora*, *Sapindus mukorossi*, *Swertia chirayita* and *Zanthoxylum armatum*. It is also noted that they have a wide distribution within the

country. Some other plants like *Acorus calamus*, *Piper chaba*, *Piper sp.*, *Rheum australe*, *Rubia manjith* and *Valeriana jatamansi* also show similar trend both in demand and in distrubution but their total collected quanitties are smaller. However the proven utility values in this group are higher than those of the former 10 items.

The private sector handles over 95% of trade through out Nepal. The major organisations that deal with the trade and processing of medicinal plants

Farmers are collecting the roots of such medicinal plants indifferently. The trader and the companies which use such products are continuously buying the products without paying any attention for the preservation of these plants and their scientific management . Community forestries of this area are concentrating their activites for the production and management of timber and firewood without due attention to the Non Timber Forest products. Therfore there exists a serious threat of depletion of valuable medicinal plants due to the lack of adequate conservation plan. The following species of MAPs are at the risk of extinction.

Endangered Medicinal Plants in Nepal

1) Acorus calamus (Sweet flag)
2) Asparagus racemosus (Wild asparagus)
3) Cordyceps sinensis (Yarsagumba)
4) Dactylorhiza hatagirea (Orchis)
5) Daiswa Dolyphylla (Satuwa)
6) Frililaria cirrhosa (Kakuli)
7) Nardostachys grandiflora (Jatamansi)
8) Picrorhiza scrophulariiflora (Kutki)
9) Podophyllum hexamdrum (Laghupatra)
10) Rauwolfia serpentina (Sarpagandha)
11) Rheum australe (Padamchal)
12) Rubia manjith (msjiyho)
13) Swertia chirayita (Chiraito)
14) Valeriana jatamansi (Sugandhawala)

<u>Research Activities in Medicinal and Aromatic Plants</u>

The following organisations are involved in R&D on MAPs.

1. Royal Nepal Academy of Science & Technology (RONAST) -

This Academy was established in 1982 with view to develop scientific capability in the country.

There is Natural Product Laboratory, under agies of RONAST which is having the research in the natural resource is doing the extraction and isolation of the taxol. RONAST is working in the field of biodiversity.

Recently a small Research and Development Project on Medicinal and Aromatic Plants have been initiated in collaboration with the Mahendra Sanskrit University (MSU). The MSU teaches Ayurvedic medicine and produces Ayurvedic Health Workes.

The overall objective of the project is to build capability of local institutions and people for the conservation and sustainable utilisation of selected MAPs that are at the risk of extinction in *in situ* conditions.
The specific objectives of the project are :

- Collection and plantation of selected MAPs such as *Rauvolfia serpentina*, *Withania somnifera* and *Cinnomomum glaucescense* etc. in the land of MSU located in Dang district of Nepal in the form of MAP biodiversity garden
- Development of cultivation packages of selected MAPs through proper evaluation and targeted research and initiation of commercial cultivation of MAPs in the land of MSU for demonstration as well as income generation purpose.
- Establishemnt of an Analytical Laboratory at Beljhundi Dang for the preliminary quality analysis of MAP products.
- Train Community Forest Users Groups to keep two way tracks of MAPs from and into their forests by providing them with the technology packages of growing MAPs in the community forestries and commercilisation of the products.

At the moment germplasms of 15 MAP specics have been collected and established in the form of a herbal garden covering 5 ha of land.

2. Department of Plant Resources

It is under the Ministry of Forest and Soil Conservation of His Majesty's Government of Nepal. It was formerly known as Department of Forestry and plant resource and even Department of Medicinal plants and it was

established in 1959 AD. Its activities are concerntrated on the detail survey and collection of Flora of Nepal and preservation of the specimens in the National Herbarium conducting research on phytochemical screening and pharmacological tests, to develop techniques of commercial cultivation of important medicianal and economic plants. It has different wings as

a. Natural Products Development Division : This division is comprises of Chemical and Technological section, Biological Research section, Instrumental section, and Pilot Plant section. Phytochemical, Pharmacological and Microbiological tests are conducted

b. Plant Research Division. It has mainly two wings viz National Herbarium and Plant Laboratories and Royal Botanical Garden which provides information on various aspects of researches on plant resources through publication.

Royal Drug Research Laboratory: This is now under the Department of Drug Administration (DDA), Ministry of Health. It has main activity to test the drug efficacy and safty before the entry to the market. Drug has to pass all the test performed by the laboratory prior to get registration in Department of Drug Administration. Ayurvedic drug , newly registered in DDA has to take the test report given by the laboratory and other test of Ayurvedic drug requested by the Ayurved Department .

Status of Industrial Utilization of MAPs - TLC Following organisation involved in processes of MAPs.

1. Singh Durbar Vaidyakhana

Singha Darbar Vaidyakhana was established approscimateby 300 years ago. From the ancient time a lot of medicinal herbs were known using in the treatment of the disease. At the early year this Vaidyakhana was serving to Royal family and high ranking people. Later it is opened to all the public. After the implementation of the Ayurvedic Policy of Nepal it is transferred to the Development Board so that it can produce more valuable product and at least in sufficient quanitty to substitute the import. About 100 types of its products are in the market. It has its sales room as well as Ayuvedic Department buys and distributes to the centres in the whole nation.

2. Herbs Production and Processing Co. Ltd (HPPCL)

It was established in 1981 as an undertaking of HMG/NEPAL. The company produces essential oils and medicinal extract using indigenous Himalayan herbs as raw material. It cultivates, collects and processes medicinal and aromatic plants. At present this company is capable of exporting indigenous products like Lichen rosinoid, Sugandha Kokila oil, and Jatamasi Oil as well as products from introduced vaieties of plants such as palmarosa oil, citronella oil, lemongrass oil. It also encourages and provides information the farmers to cultivate the medicinal plants.

3. Dabur Nepal - This is the sister organisation of Dabur India co. Ltd. recently established. It collects and processes mainly tasol from Tasus baceata.

4. Gorkha Ayurved Company Ltd. - This is a private Company producing different by cerrecdic medicines form the MAPs.

Besides these there ere 15 Ayurvedic Manufacturers that are processing the medicinal plants in the country. There are numerous local medical practioners in the country utilising medicinal herbs for the treatment of local people.

COUNTRY REPORT

COUNTRY: Pakistan

NAME: Dr. Farzana Shaheen

COUNTRY REPORT

STATUS OF MEDICINAL PLANT RESEARCH IN PAKISTAN

PRESENTED BY

Dr. Farzana Shaheen

IMPORTANCE OF MEDICINAL PLANTS IN BASIC HEALTHCARE

PAKISTAN SCENARIO

1. An estimated 80% of the rural population of Pakistan depends on traditional medicines for their primary healthcare need, majority of which use plants of their active principles.
2. There are around 5,000 species of wild plants in Pakistan.
3. According to the National institute of health (N.I.H.) about 400 plant species are used in traditional medicines.
4. Traditional healers around 50,000 are serving about 60% of the population, specially those living in the rural areas.
5. Tibbi pharmacopoeia (pharmacopoeia of Traditional Drugs compiled by the Tibbi Board) has listed around 900 single drugs and about 500 compound preparations made out of medicinal plants.
6. There are about 27 large herbal manufacturing companies in Pakistan which product Unani medicines on commercial scale. The number of herbal medicine manufacturers in non-organized sector run into hundreds.
7. The annual turnover of some large herbal manufacturers is comparable to multinational companies in Pakistan.

INSTITUTIONS INVOLVED IN MEDICINAL PLANT RESEARCH IN PAKISTAN

A MEDICINAL PLANTS

Phytochemistry

- International Centre for Chemical Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi.
- Pakistan Council of Scientific and Industrial Research (P.C.S.I.R.) Laboratories, Peshawar, Pakistan.
- Chemistry and Pharmacology Departments of Various Universities.

Taxonomy, Collection, Herbarium

- Pakistan Forest Institute, Peshawar.
- National Agricultural Research Council (NARC) (Plant Genetic Research Centre and National Herbarium), Islamabad.
- Herbarium, University of Karachi, Karachi.
- Hamdard University, Karachi (to a very limited extent).
- Botany Departments of Various Universities.

Pharmacology

- HEJ Research Institute of Chemistry, University of Karachi, Karachi.
- The Aga Khan Medical University, Karachi.
- Hamdard University, Karachi.
- National Institute of Health, Islamabad.

- University of Agriculture, Faisalabad (veterinary)
- Pharmacology and Pharmacognosy Departments of Various Universities.

Standardization, Quality Control and Safety Assurance of Plant-Based Drugs

- Hamdard University, Karachi.
- Pharmacy Faculty, University of Karachi, Karachi.
- HEJ Research Institute of Chemistry, University of Karachi, Karachi.

Medicinal Plant Cultivation

- Pakistan Forest Institute, Peshawar.
- Hamdard University, Madinatul Hikmat Campus, Karachi.
- Experimental Farm at the National Agricultural Research Council, Islamabad.
- Some Small Farms in Private Sector.

Tissue-Culture

- National Agriculture Research Council, Islamabad
- Department of Botany, University of Peshawar
- H.E.J. Research Institute of Chemistry, University of Karachi
- N.I.B.G.E., Faisalabad

Ethanobotany, Database, Pharmacoepia

- Pakistan Forest Institute, Peshawar
- Department of Botany, Quaid-i-Azam University, Islamabad
- Department of Botany, University of Balochistan, Quetta
- Baitul Hikmat Research Institute, Hamdard University, Karachi
- Department of Botany, University of Peshawar

INSTITUTIONS INVOLVED IN MEDICINAL PLANT RESEARCH IN PAKISTAN

B MARINE NATURAL PRODUCTS

Chemistry

- HEJ Research Institute of Chemistry

Taxonomy

- National Institute of Oceanography, Karachi
- Center of Excellence in Marine Sciences University of Karachi
- Marine Reference Collection and Reference Center, University of Karachi

PROFILE OF THE HEJ RESEARCH INSTITUTE OF CHEMISTRY
(INTERNATIONAL CENTER FOR CHEMICAL SCIENCES)

- **Established in**: 1965 as a part of the Department of Chemistry, University of Karachi.

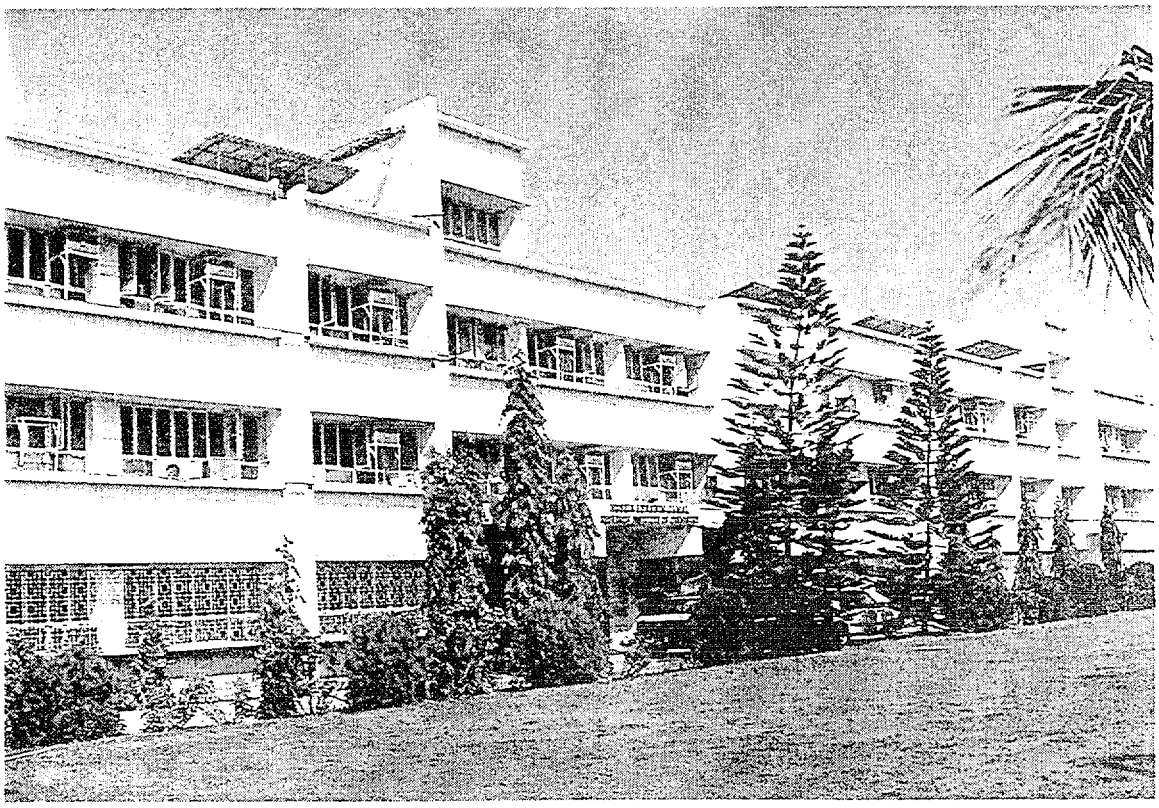
- **Upgraded as**: the Third World Center for Chemical Sciences in 1997 and several new buildings were added.

- **Students**: the single largest doctoral program, about 120 students are enrolled for M.Phil. and Ph.D.

- **International Visitors**: from Germany, Cameroon, Mongolia, Nigeria during 1999-2000.

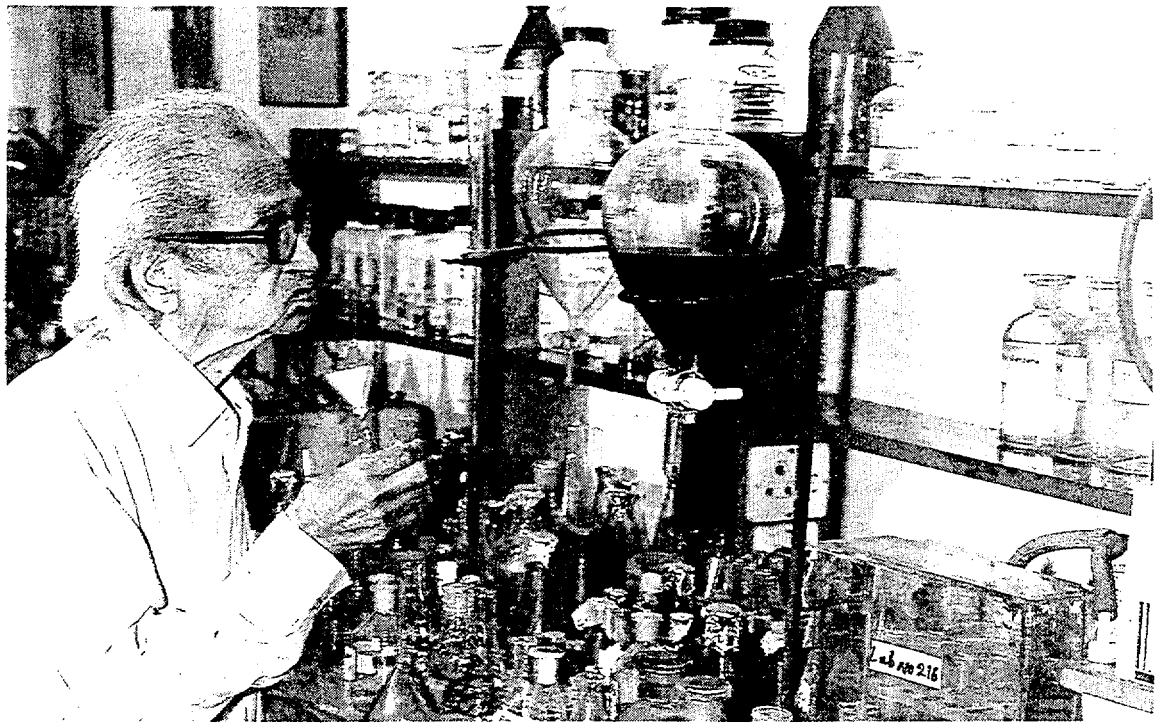
- **Research work**: Structural chemistry, Bioassay directed isolation of bioactive compounds, Organic synthesis of drug molecules, X-ray crystallography, Microbial transformations of bioactive natural products and Chemical derivatization of important natural products.

Husein Ebrahim Jamal (H.E.J.)
Research Institute of Chemistry
INTERNATIONAL CENTER
FOR CHEMICAL SCIENCES



University of Karachi, Karachi-75270, Pakistan
Telephone: (92-21) 496-8497, 496-8498, 496-8733,
496-8926, 499-0007
Telefax: (92-21) 496-3373, 496-3124
E.Mail: hejric@biruni.erum.com.pk
hej@biruni.erum.com.pk
Home Page: www.comsats.net.pk/~heji

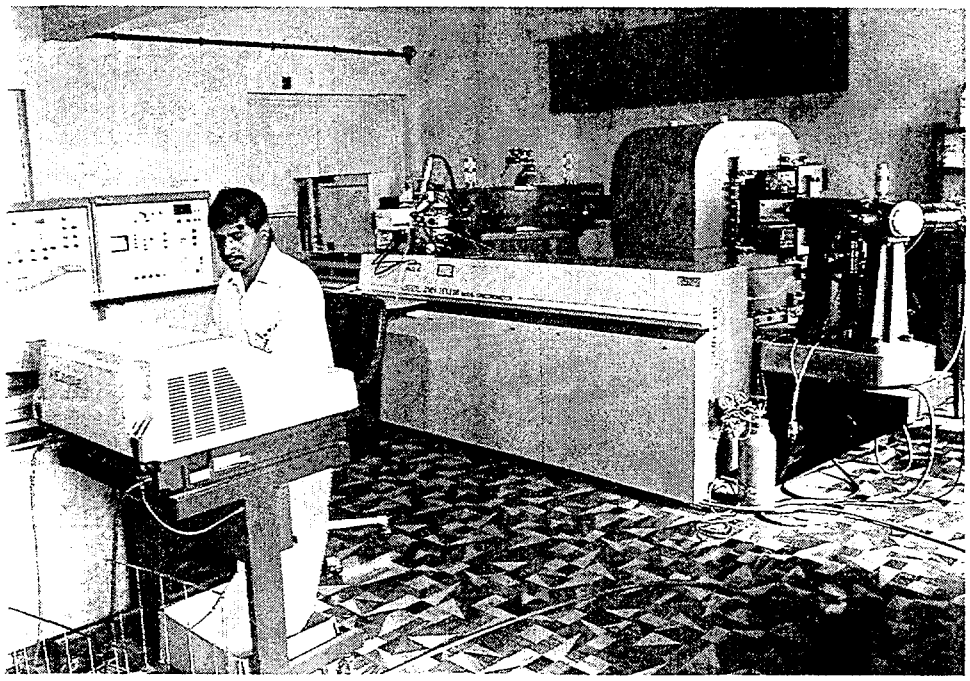
November, 1997



LATE PROF. SALIMUZAMAN SIDDIQUI, *H.I., F.R.S.*
Founding Director
H.E.J. Research Institute of Chemistry

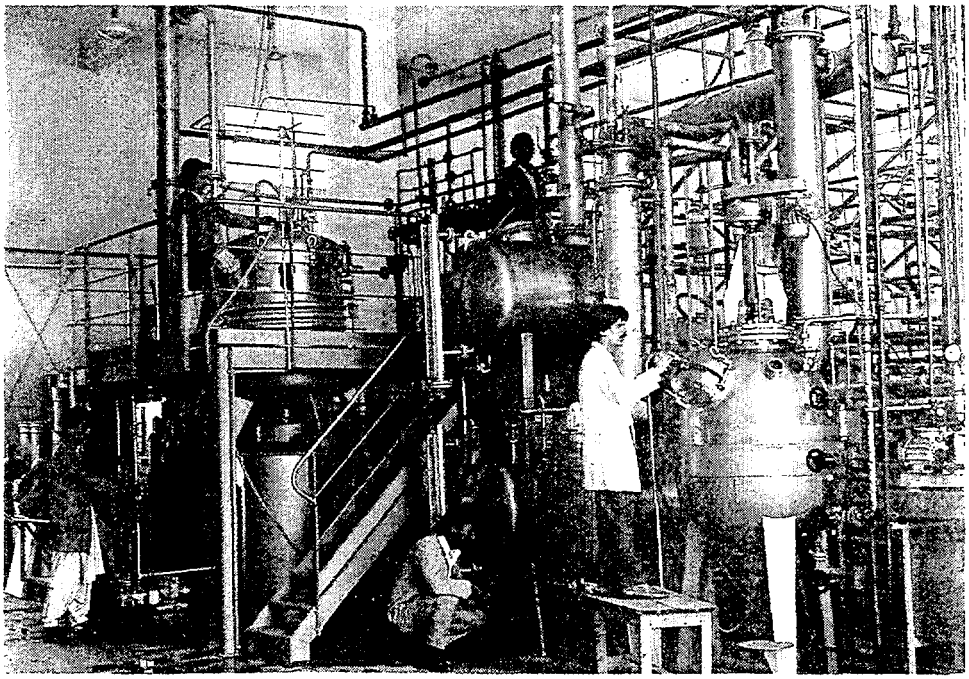
PROF. ATTA-UR-RAHMAN, *H.I., S.I., T.I.*
Director
H.E.J. Research Institute of Chemistry

A BRUKER AMX 500 (500 MHz) NUCLEAR MAGNETIC RESONANCE SPECTROMETER (ONE OF TWO)
(Mr. M. Sarfarazullah)



JEOL HX 110 HIGH RESOLUTION MASS SPECTROMETER WITH FAB, GC AND FD SOURCES
(Mr. Yaqoob John)

ANALYTICAL LABORATORY
(Mr. Touheed Ahmed)



PILOT PLANT SECTION
(Mr. Intikhabul Haq)

INTRODUCTION

A nucleus of the Institute was established under the Directorship of late Prof. Salimuzzaman Siddiqui, H.I., F.R.S. in 1967 on one of the floors of the Department of Chemistry. Prof. Atta-ur-Rahman joined the Institute initially in 1969. He was then elected as a Don at Kings College, Cambridge University, and after completing a 4 year stay in Cambridge University as Fellow of the Kings College, he re-joined the institute permanently in 1973 and was appointed Co-Director in 1977. A number of projects were prepared and submitted to foreign-aid giving agencies, which were funded to the tune of 4.8 million DM from Germany, 1 million pounds from the U.K., 8 million dollars from Japan, 3.5 million DM from Germany and, more recently, 3.0 million dollars from U.S.A. which have transformed the institute to one of the finest centres of natural product chemistry in the world. The dedicated efforts of the faculty members, students and employees of the institute played a vital role in these developments.

Having the single largest doctoral program in the country, the institute provides a place of work to about a hundred bright young scientists who are enrolled for Ph.D. level studies on various aspects of organic chemistry, biochemistry and pharmacology. Pakistan produces about 30 Ph.D's in the sciences annually from its 24 universities and 130 research centers, of which more than half are now produced by H.E.J. Research Institute of Chemistry alone.

A number of goal-oriented projects relating to the chemistry of natural products and protein chemistry are being vigorously pursued which have led to the award of over 100 doctorate degrees, 30 M. Phil. degrees and 35 M.Sc. degrees and the publication of over 800 research papers that have earned international recognition.

The areas of research covered in the programs of the institute broadly relate to isolation, structural, synthetic and pharmacological studies on novel natural products as well as various aspects of protein chemistry. To get a clearer idea of the wide range and orientation of the basic researches carried out at the institute would need careful reference to the topics covered in over 800 research publications in international journals. In order to ensure international standards the doctorate degrees are awarded to students of the institute on the recommendations of two eminent scientists from abroad after their assessment of the doctoral theses referred to them. The scientists trained in the institute are now serving the country in industry and in various R & D and academic institutes. The quality of the researches being pursued in the institute are reflected from the fact that all the Professors in the institute have been awarded D.Sc. degrees (Prof Salimuzaman Siddiqui: Leeds University; Prof. Atta-ur-Rahman: Cambridge University; Prof. V.U. Ahmed: Karachi University and Prof. Zafar H. Zaidi: Leeds University), a unique achievment for a research institute in a Third World country.

Various collaborative researches have been undertaken jointly with scientists of the developed as well as Third World countries including those with Prof. Al Sayed Alashry (Egypt), Prof. Azad Choudhury (Bengladesh), Prof. Jon Clardy (USA), Prof. Ermias Dagne (Ethiopia), Prof. K.T. DeSilva (Sri Lanka), Prof. Victor Fajardo (Chile), Prof. Leslie Gunatilaka (Sri Lanka), Prof. Jim Hanson (U.K.), Dr. W.H.M.W. Herath (Sri Lanka), Dr. Masood Parvez (Canada), Dr. David Rycroft (UK), Prof. Salim Sabri (Jordan), Prof. Bilge Sener (Turkey), Prof. Maurice Shamma (USA), Prof. Asifuzzaman Siddiqui (India), Dr. David L. Smith (USA), Prof. Wolfgang Voelter (Germany) and Prof. Bing Nan Zhou (China), which have led to exciting new results published in leading international journals. The Institute is also one of the few research institutes in the Third World where students from Western countries are coming for training in sciences.

MR. LATIF EBRAHIM JAMAL
Chairman,
Husein Ebrahim Jamal Foundation

PROF. WOLFGANG VOELTER

MR. JAMIL AHMED KHAN
A Philanthropist

HISTORY

On the retirement of Prof. Salimuzzaman Siddiqui as Chairman, PCSIR, his services were taken up by the University of Karachi and a "Postgraduate Institute of Chemistry" was established under his Directorship in 1967 in a wing of the department of chemistry. PCSIR provided the services of some of its staff (including Dr. Viqar Uddin Ahmad and Dr. Zafar H. Zaidi) and furniture during the initial phase of the establishment of this institute. Dr. Atta-ur-Rahman joined the Institute in March 1969 after obtaining Ph.D from Kings College, Cambridge, Later he accepted a 4-year assignment as a Fellow of Kings College, Cambridge University. He was then sent by Cambridge University to the University of Karachi during 1970/71 to assist in the setting up of a modern postgraduate institute of chemistry, and he brought with him donations of gas chromatographs, balances and a combustion microanalyser from Cambridge University. He succeeded in obtaining grants to acquire a new mass spectrometer and an NMR spectrometer both of which were installed in 1974, within a year of his return to Pakistan.

Prof. Atta-ur-Rahman joined the institute permanently in 1973 and he was appointed as Co-Director in 1977. He succeeded in winning several major projects for the institute from the West (1 million pounds from U.K., 8 million dollars from Japan, 3.5 million DM from Germany etc. and more recently a 10 million U.S. dollars project has been approved by the Government of Pakistan for the establishment of the International Centre for Chemical Sciences) which have transformed the institute into the finest centre in Asia and one of the best in the world in the field of natural product chemistry. Prof. Atta-ur-Rahman was appointed as Director in January 1990, while Prof. Viqar Uddin Ahmad took over as Co-Director in 1990 and was appointed as the Dean, Faculty of Science in 1994 while continuing as Co-Director in the institute.

The efforts of Prof. W. Voelter of Tubingen University, Germany, deserve special praise since the initial grant of 2.3 million DM from the Federal Republic of Germany was largely due to his dynamic efforts, and collaborative researches continue with his group. A number of goal-oriented projects relating to the chemistry of natural products were vigorously pursued which led to the award of a number of doctorate degrees and the publication of numerous research papers that earned international recognition. In appreciation of these contributions a generous donation of Rs. 50 lacs was offered to the University for the Institute by the leading philanthropist/industrialist Mr. Latif Ebrahim Jamal on behalf of the Husein Jamal Foundation in 1976, the largest donation at the time in the history of the country. The Institute was accordingly named as Husein Ebrahim Jamal Research Institute of Chemistry in memory of the late Mr. Husein Ebrahim Jamal, the elder brother of Mr. Latif Ebrahim Jamal. Rs. 2.5 million from the donation amount was used for the construction of the main building of the institute, to which it shifted in summer 1978, while the remaining Rs 2.5 million was invested in the shares of the Husein Ebrahim Jamal Foundation.

Recently a 10 million dollar project submitted by Prof. Atta-ur-Rahman for the establishment of International Centre for Chemical Sciences has been approved

for the Institute by ECNEC in October 1994. New areas of frontier research will be added in the institute and the institute will provide training to scientists from other Third World countries.

The Institute has organized a number of major international conferences and symposia on various aspects of natural product chemistry, spectroscopy and protein chemistry. International symposia on spectroscopy, natural products and protein chemistry are held every year in Karachi and they have attracted eminent chemists (including several Noble Laureates) from all over the world and have become renowned as among the best conferences in the field. The institute had the distinction of hosting the 19th IUPAC Symposium on Natural Product Chemistry in January 1994 in which over 500 scientists from 52 countries including 3 Nobel Laureates participated.

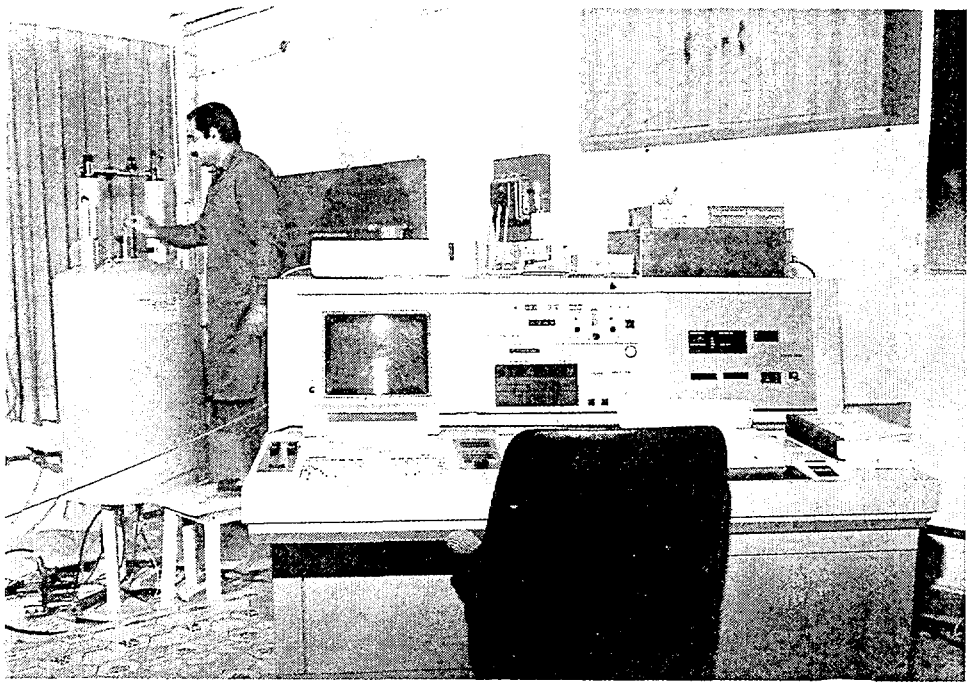
The H.E.J. Research Institute of Chemistry has recently been selected as the venue for the International Centre of Science and Technology in Chemical Sciences. A number new fields such as medicinal chemistry, agricultural chemistry, protein crystallography, genetic engineering and computational chemistry are proposed to be established along with the expansion of existing areas of research. The H.E.J. Research Institute of Chemistry has also been selected as one of the three library centres which will be established by the Third World Academy of Science (TWAS) in its member countries.

H.E.J. Research Institute of Chemistry has been designated as the W.H.O. Centre for Pesticide Analysis for the Eastern Mediterranean Region. Routine analysis of the pesticide and insecticide samples from W.H.O. will be carried out in the analytical laboratories of the Institute in the near future along with researches in the development of new safer pesticides.

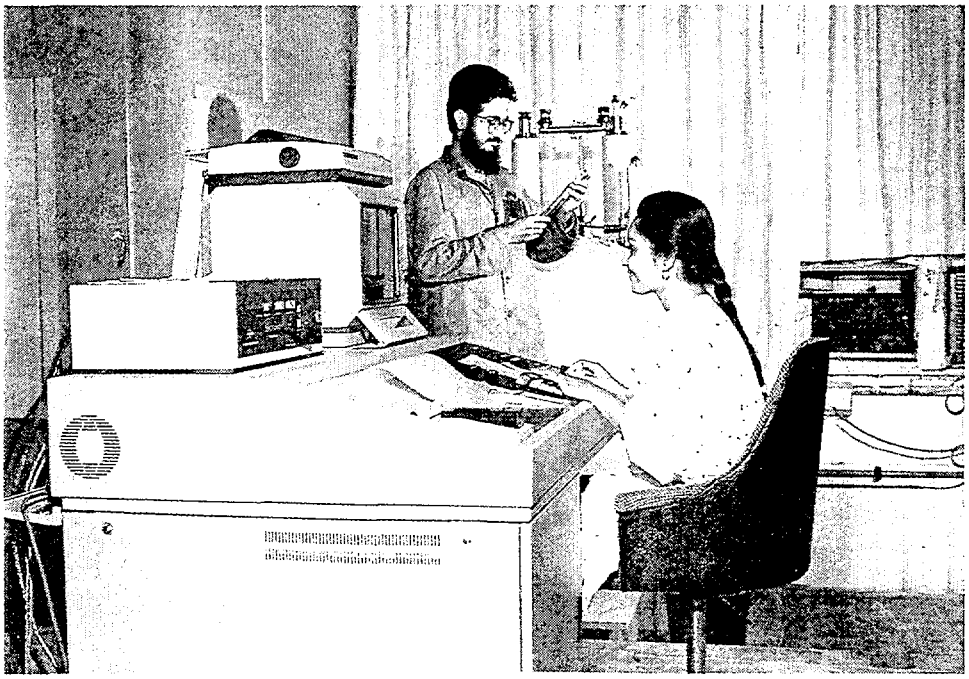
Mass spectroscopic data bases, ORAC (Organic Reaction Access), ISI current content, dictionary of natural products and LHASA data bases have recently been acquired by the Institute. A new MicroVax 3100 computer system and Silicon Graphic Work Station along with graphic and printing facilities have already been installed. Several laboratories such as tissue-culture, plant micropropogation, antitumor and radiolabelling laboratories have been established in the collaboration with NCI (USA), Abbott Labs. (USA) and others.

USAID has also supplied a large gas-fired diesel generator system, and funded a number of research projects at H.E.J. Research Institute of Chemistry. These projects are designed to promote industry/academia collaboration in areas of applied nature.

A network link will soon be established with the help of IBM (Paksitan) which will upgrade the existing dial-up connection into a full INTERNET node.



BRUKER AM-400 (400 MHz) NMR SPECTROMETER
(Mr. Gul Rahim)



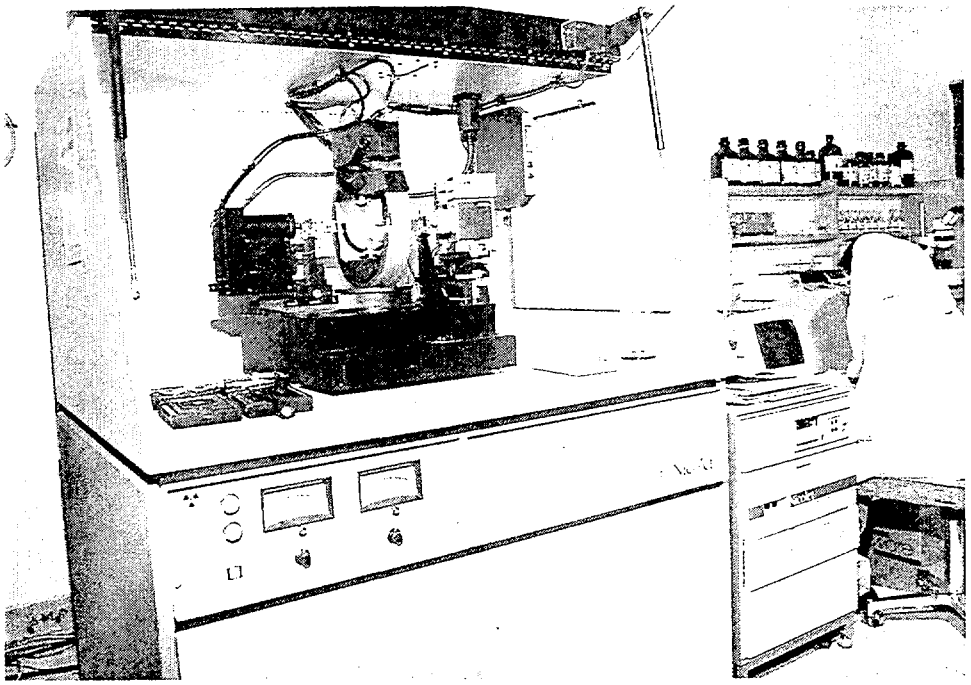
BRUKER AC-300 (300 MHz) NMR SPECTROMETER
(Ms. Zohra Jabeen)

FACILITIES

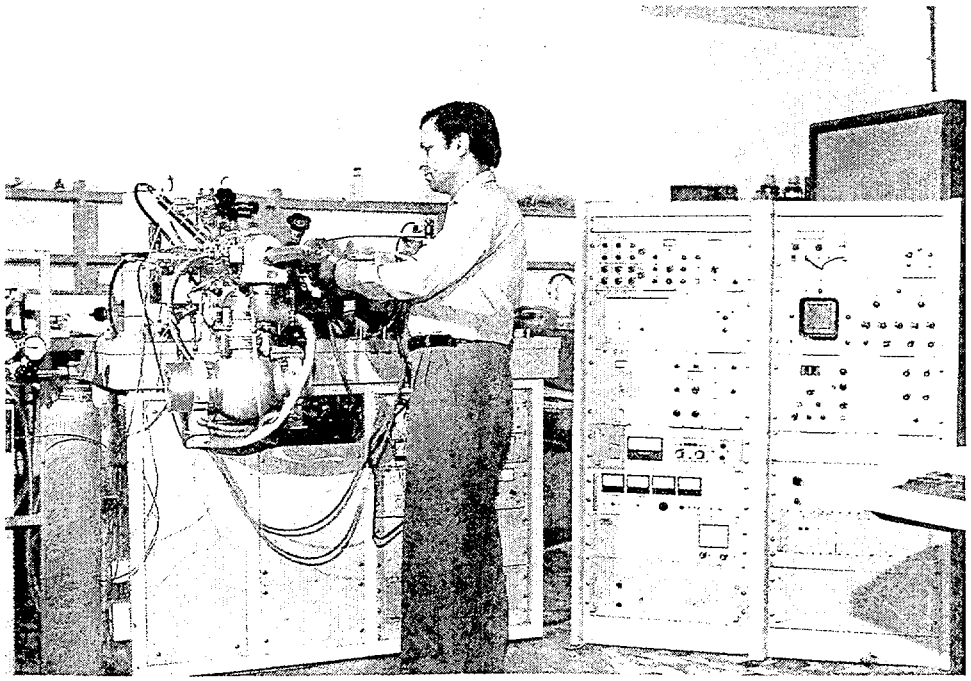
The equipment installed in the Institute includes X-Ray diffraction and molecular modelling facility with a single-crystal X-Ray diffractometer (R3M/V, Siemens) connected to a MicroVax II computer system with Tektronix graphic display. The high field nuclear magnetic resonance (NMR) spectroscopic laboratories of the institute include five state-of-the-art superconducting instruments (two 500 MHz, one 400 MHz, two 300 MHz Bruker pulse NMR spectrometers). This is one of the most sophisticated NMR facilities in the world and the largest in Asia. Most of the latest 2D and ID pulse NMR experiments are routinely performed in the NMR laboratories of the institute. The mass spectroscopic laboratories of the institute contain six mass spectrometers of which five are high resolution double focussing instruments (Jeol HX 110 G.C.-M.S., Varian MAT 312, Varian MAT 311 G.C.-M.S., Varian MAT 311A and Varian MAT 112, G.C.-M.S.) linked to two DEC PDP 11/34 computer systems. A small VG mass spectrometer is generally used for training purposes. These instruments are performing a wide variety of mass spectroscopic experiments including negative and positive fast-atom bombardment, chemical ionization, field desorption, field ionization and electron-impact (high resolution) experiments. Other major instruments include a gas phase amino acid sequencer (Applied Biochem), amino acid analyzer (Biotronik), FT infrared spectrophotometer (FTS-65, Biorad), IR spectrophotometers (IR-460 Shimadzu, IRA-1 Jasco, IRA-302 Jasco), UV spectrophotometers (Shimadzu UV-240, Hitachi U-3200), gas chromatographs (GC-9A Shimadzu, GC-6800 Dani), high pressure liquid chromatographs (more than 18 different systems including Shimadzu, Waters, Beckmann, LDC etc.), CD spectropolarimeter (J-600 Jasco), polarimeters (DIP-360 Polatronic-D and Jasco), elemental analyzer (MOD-1106 Carlo Erba and Corder-MT-3 Yanaco), helium and nitrogen liquefication systems (Sulzer), cold rooms, large scale pilot plant extraction set-up (Tournaire), glass blowing section, pharmacological testing instruments such as polygraphs, β-counter (LKB), γ-counter (LKB) etc. Uninterruptable power supply to these very sophisticated instruments is guaranteed by two UPS systems connected to both external power supply and in-house electricity generating systems.

The instruments are maintained by a team of competent electronic engineers comprising Mr. Javaid Iqbal Shaikh, Mr. Abdus Sami, Mr. Mazhar Jameel Khan and Miss Shabana Ikram. The electronic engineers and other technical personnel include Mr. M. Sarfrazullah, Mr. Yaqoob John, Mr. Barkat Ali, Mr. Jamil Ahmad, Mr. Tariq Sami and Mr. Touheed Ahmad who have been trained abroad on the various sophisticated instruments installed in the Institute.

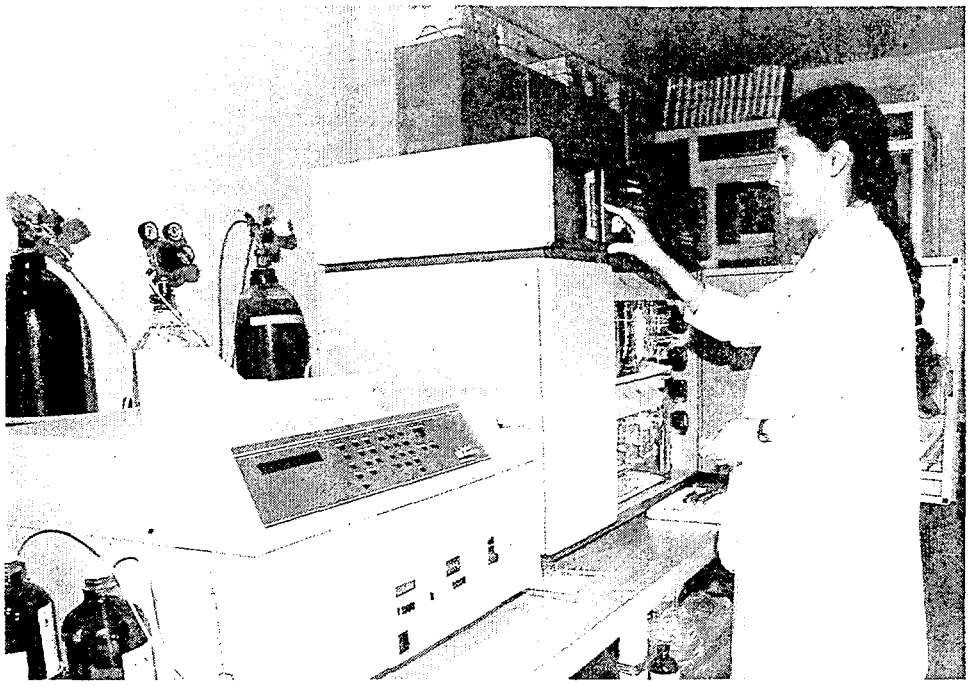
The Institute has been offering an instrumentation service to the various universities and research centers of Pakistan. The spectroscopic services have also been extended to collaborating scientists of various countries such as Bangladesh, Chile, Ethiopia, Egypt, Ghana, India, Iran, Iraq, Jordan, Mongolia, Sri Lanka, Turkey, etc.



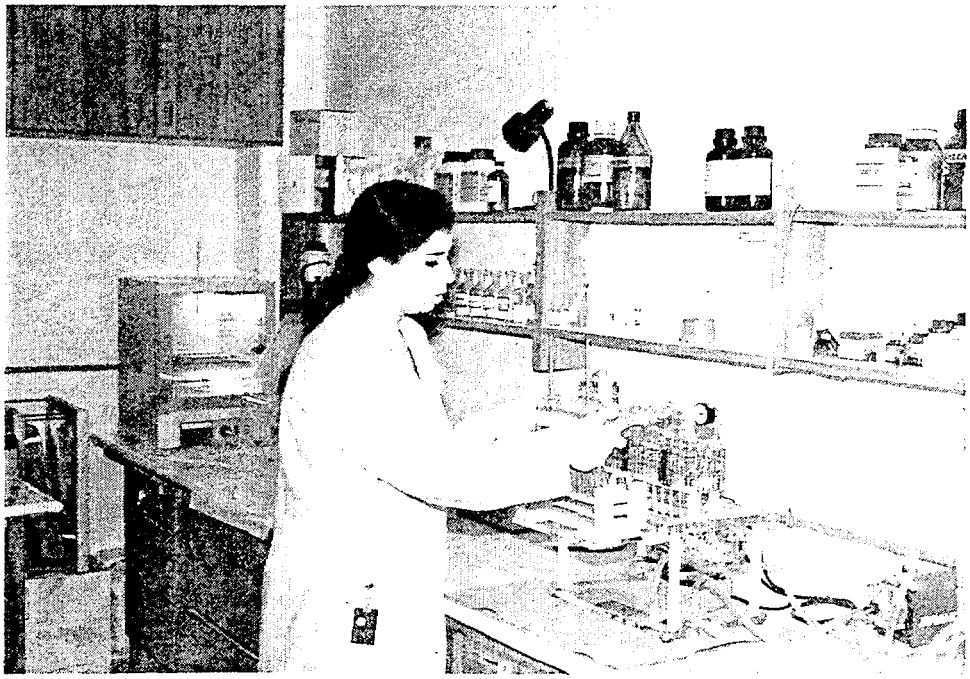
NICOLET (NOW SIEMENS) R_3M/V SINGLE-CRYSTAL X-RAY DIFFRACTOMETER WITH MICROVAX II COMPUTER SYSTEM



FINNIGAN MAT 312 MASS SPECTROMETER
(Mr. Barkat Ali)



APPLIED BIOCHEM GAS PHASE AMINO ACID SEQUENCER
(Dr. Atiya Abbasi)



LABORATORY FOR RECEPTOR BINDING ASSAYS
(Miss Lubna Hameedun Nisa)

RECENT DEVELOPMENTS AND FUTURE PLANS

The Third World Academy of Sciences at its meeting held on Oct. 15, 1990 in Caracas, Venezuela took the historic decision of setting up 20 large International Centres for Science and High Technology so that the Third World countries could compete with the advanced Western nations in key scientific fields and catch up in critical areas of technology.

The most important recent event is the selection of H.E.J. Research Institute of Chemistry as the International Science and Technology Centre for Chemical Sciences after stiff international competition. Two European experts, Prof. Wolfgang Voelter of the University of Tubingen, Germany and Dr. David Walton of the University of Sussex, U.K. were appointed by the Third World Academy of Sciences in July 1994 to carry out an independent assessment of the Institute regarding its research output, performance and potential to be developed as a full-fledged Centre of Science and Technology in Chemical Sciences. Their report formed a strong justification for the selection of the Institute as the first Third World Centre for Science and Technology in Chemical Sciences. Pakistan is now presented with a unique opportunity of having one of the finest World Centres in a frontier field of science and technology. The Prime Minister chaired a meeting of COMSATS (Commission on Science and Technology for Sustainable Development in the South) on 4th and 5th October, 1994 which was attended by Ministerial level representatives of some 40 Third World Countries. Inspite of alternative proposals and vigorous efforts from China, Brazil, India and other Third World Countries it was agreed in the meeting that the first International Center for Science and Technology would be established at the H.E.J. Research Institute of Chemistry of Karachi University. A project for the development of H.E.J. as the International Centre was also approved by ECNEC at a cost of Rs. 336 million and the project commences on 1st July, 1995.

The Executive Committee of the National Economic Council (ECNEC) approved the project for the establishment of the International Centre by expernation of H.E.J. Research Institute of Chemistry on Oct. 18, 1994 at a cost of Rs. 331 million with a foreign exchange component of Rs. 276 million. Pakistan is now presented with a golden opportunity of establishing a really large science centre in the field of chemical sciences.

The industry-related projects to be conducted in the Centre will provide a significant boost to be local industry and the applied nature of the research programs will have a good impact on the economic development of Pakistan Chemistry plays a vitally important role in industry and its wide ramifications can be seen from its applications in the fields of medicine (pharmaceuticals), agricultural sciences (insecticides, pesticides, fertilizers, etc.), polymer sciences (synthetic fibers, plastics, paints), cement industry, glass technology, iron and petroleum industry, ceramics, soap and detergent, leather, textile, etc. In short, it would be fair to state that there is no other single scientific discipline of more significance than chemistry in national development since it has an important role to play in almost every facet of industrial development.

ACADEMIC CONTRIBUTIONS

A brief summary of the contributions of the faculty members of the Institute in the form of Ph.D.s produced, research publications and books written or edited is given below:-

Name of Scientist	Research Publications	Books	Chapters in Books	No. of Students Completed Ph.D./ M.Phil Degree
Prof. Atta-ur-Rahman	295	43	48	40
Prof. Viqar Uddin Ahmad	223	6	8	20
Prof. Zafar H. Zaidi	100	7	7	19
Prof. Bina S. Siddiqui	172	-	18	4
Dr. M. S. Shekhani	34	-	-	3
Prof. Abdul Malik	115	-	-	7
Dr. M. Iqbal Choudhary	109	5	28	3
Dr. Atiya Abbasi	45	2	18	-
Dr. Shaheen Faizi	77	-	-	1
Dr. Sabira Begum	79	-	-	-
Dr. Humayun S. Ateeq	20	-	-	-
Dr. Ahsana Dar	5	-	-	-
Dr. Darakhshanda Shehnaz	4	-	-	-
Dr. Shaiq Ali	58	-	-	-

COUNTRY REPORT

COUNTRY: Phillipines

NAME: Dr. Ritche Manos Hao

Country Report

Herbal Medicine Research in the Philippines

presented at the

"Training Course on Research Strategies on Medicinal and Aromatic Plants"

August 14-18, 2000
Bangkok, Thailand

Presented by:

Ritche M. Hao, MD
Country representative

Country Report

Herbal Medicine Research in the Philippines

I. Introduction

The Philippines has a rich tradition of herbal medicine use. From traditional healers in the countryside to the housewife who concocts remedies from her backyard garden, the use of plants for medicinal purposes remains an alternative to traditional healing practices. This rich tradition dates back centuries and has been handed down from generation to generation. Although seemingly taken over by the onslaught of modern scientific healing techniques, the practice of herbal medicine in the Philippines continues to flourish.

Because the cost of healthcare continues to escalate and the rise of drug prices remains unabated, alternative modes of treatment have been sought out both by the government and non-government sectors. A practical solution to this problem is to tap natural remedies that are already in use by many Filipinos. By scientifically validating these remedies, reliable yet economical ways of treating disease can be made available to the public at large.

At the onset, researchers in the Philippines have individually attempted to scientifically validate the use of certain herbal concoctions for the treatment of specific diseases. In general, the output of these researches have not been fully tapped as there was no venue where such knowledge could be consolidated and be put to practical use. It was only in 1974 when a group of researchers from different sectors and institutions organized themselves and put up the National Integrated Research Program on Medicinal Plants (NIRPROMP), a research body which aimed to systematize the study of medicinal plants in the Philippines with an end goal of providing safe, effective, and affordable pharmaceutical products derived from commonly available plants.

To this date, a number of plants have already been studied and proven to be safe and effective. Pharmaceutical dosage forms of some of these plants have already been marketed and sold to the general public as affordable alternatives to traditional drugs. Scientific validation of newly identified plants are also underway. Apart from NIRPROMP, many other institutions are now taking interest in herbal medicine research. Although still a long way to go, herbal medicine research in the Philippines continues to thrive and will hopefully pave the way for more affordable medicines for Filipinos.

II. Herbal Medicine Research in the Philippines: Current Status

A. National Integrated Research Program for Medicinal Plants (NIRPROMP)

The National Integrated Research Program for Medicinal Plants (NIRPROMP) was established in 1974. Overseen by the Philippine Council for Health Research and

Development of the Department of Science and Technology, the NIRPROMP was organized to spearhead herbal medicine research in the Philippines. It involved institutions like the University of the Philippines Colleges of Agriculture, Science, Medicine, and Pharmacy; the National Institute of Science and Technology; and the Department of Health, Education, Culture, and Sports, and Agriculture.

The need for more systematic ways of studying medicinal plants was the impetus for the creation of the NIRPROMP. It had as goal the need to provide affordable medicines for the majority of Filipinos. A secondary goal was to discover novel or better cures for diseases.

Whereas the initial goal of earlier herbal medicine researchers was to find the active principle responsible for the plants effectivity in curing diseases, this proved to be cumbersome and was deemed too costly. Thus, it was incumbent for the NIRPROMP not only to prove the safety and efficacy of medicinal plants but also to find easier and more cost effective ways of using such plants. Although finding the active principle of the medicinal plant would be the eventual goal, the use of whole plant parts provided for easier, faster and more cost efficient ways of exploiting the medicinal value of identified plants.

Initial research activities of the NIRPROMP consisted of verifying the folkloric claims for identified plants by using basic pharmacologic techniques and rapid clinical screening methods. Data on plants proven safe and effective (eg, indications for use and methods for preparation, for example, as decoction, infusion, juice, or poultice) were then conveyed to health workers at the community level for dissemination to their constituents.

The data gathered from these studies were also compiled and published in guidebooks on the proper use of medicinal plants. These guidebooks have now been adapted by the Department of Health, Department of Education, Culture and Sports, and various health NGOs and have helped to promote herbal medicine use in the rural areas.

At present, there are 10 plants which have been identified for such purposes. These plants are well studied and have passed safety and efficacy tests. Preparation and use of such medicinal plant products can easily be done at home, thus providing safe, effective, and cost effective alternative modes of treatment.

Production of commercially prepared drug forms (eg as tablets, syrup, ointment, or lotion) for these plants is also a goal for NIRPROMP. Through the years, the NIRPROMP has already transferred the technology of drug preparation for 3 identified plants to local pharmaceutical companies. Drugs derived from these plant products are now commercially available at popular prices. More plants are currently being studied. The expected output of commercial production of these plant products, should provide cheaper alternative drugs for the masses in the future.

At the outset, the NIRPROMP will spearhead the identification of the active principle of the plants already available for commercial use. In depth phytochemical and pharmacologic studies will be needed. This will entail a long, tedious, and costly process. The help of the private sector will surely be needed once the projects are underway.

In the meantime, more plants are being screened for possible inclusion in pharmacologic and clinical studies. The NIRPROMP continues to be unrelenting in this respect.

Timeline of Herbal Medicine Research in the Philippines 1991-2000

1991	Clinical trials revealed the effectiveness of *sambong* (*Blumea balsamifera* (L)) in treating urolithiasis and *akapulko* (*Cassia alata* L) as an antifungal
1992	Clinical trials confirmed the efficacy of *lagundi* (*Vitex negundo* L) as a cough remedy
1993	Research guidelines for the evaluation of safety and efficacy of herbal medicines were developed by the (World Health Organization) WHO
1994	The Department of Science and Technology and the Department of Health launched the akapulko lotion as an antifungal The Bureau of Food and Drugs developed guidelines for registering medicinal products for those who will adopt NIRPROM technologies
1995	Transfer of production technology for *lagundi* tablet as cough remedy to Pascual Laboratories, Inc.
1996	Herbal products *lagundi* (Ascof) and *sambong* (Re-leaf) were introduced to the market by Pascual Laboratories
1997	Ascof and Re-leaf won the Silver medal (medicine category) at the 25th International Exhibition of Inventions, New Techniques and Products held in Geneva in April 1997 Pascual Laboratories was given the Golden Shell Award Rising Star citation for innovative products with huge potentials in the international market. The Philippine Institute of Traditional and Alternative Health Care was created under Republic Act 8423 with herbal medicine research and development as one of its prominent features. Guidelines for the appropriate use of herbal medicines was developed by the WHO.
1998	Transfer of technology to the private sector was done for *akapulko* lotion and *lagundi* syrup NIRPROMP strengthened herbal medicine program in the country's main island groups Completion of preclinical studies on mahogany seeds (*Swietenia macrophylla* Jacq)
1999	List of new priority plants finalized
Present	Clinical trials of dosage formulations of *tsaang gubat* (*Ehretia microphylla*) as antispasmodic, *yerba buena* (*Mentha cordifolia Opiz* ex *Fresen*), *ampalaya* (*Momordica charantia*) for diabetes. Preclinical studies of new plants possibly effective for malaria, dengue fever, and tuberculosis are also currently underway.

B. Philippine Institute of Traditional and Alternative Health Care

Republic Act No. 8423 signed into law in 1997 created the Philippine Institute of Traditional and Alternative Health Care (PITAHC). With the creation of PITAHC, it is hoped that the development of traditional and alternative health care in the Philippines will become unhampered. A development fund has been appropriated for the activities of PITAHC. The objectives of this Act, also known as the Traditional and Alternative Medicine Act (TAMA) of 1997, include the following: to encourage scientific research on and to develop, promote, and advocate the use of traditional and alternative health care systems which have been proven to be safe and effective. Among the programs covered by the TAMA is the use of herbal medicine.

At present, PITAHC has supported the efforts of NIRPROM by publishing the manuals on the use of 10 plant medicines. It has also provided financial support for many researches on herbal medicines such as the ethnomedical documentation of medicinal plants conducted by the Complementary and Traditional Medicine Program of the National Institutes of Health, University of the Philippines Manila.

PITAHC coordinates with both the government and nongovernment sectors in the pomotion of herbal medicine research in the Philippines.

C. Others

Herbal medicine research in the Philippines is in its burgeoning phase. Of late, many other institutions have become interested in the promotion of herbal medicine research. At the forefront are the leading universities in the country such as the University of the Philippines, University of Santo Tomas, Ateneo de Manila University and De la Salle University. With the success of the commercialized plant dosage forms, many other pharmaceutical companies have expressed interest in herbal medicine research. Health nongovernmental organizations are also taking part in this effort.

III. Herbal Medicine Research in the Philippines: Future directions

At present, the NIRPROMP, being at the core of herbal medicine research, continues with its efforts at proving safety and efficacy of medicinal plants. Pharmaceutical dosage forms are continuously being prepared from various medicinal plant materials. New plants are being identified and subjected to various pharmacologic/toxicologic tests and bioassay procedures. Clinical trials are also underway for a number of plants. Technology transfer protocols will also be prepared for plants which have passed safety and efficacy testing.

Various agencies and institutions such as the NIRPROMP, PITAHC, Department of Health, National Institutes of Health University of the Philippines Manila, among others continue to coordinate with each other and forge new alliances towards undertaking rich

and promising research endeavors. A wide range of activities are also being done to augment and update knowledge of researchers in the field of herbal medicine research.

In the future, the data amassed from all these research endeavors would have been used to integrate the use of herbal medicine into the national health care delivery system. It is hoped that with such knowledge, safe, effective, and cost efficient modes of treatment will be available to the general public. The realization of this goal may still take some time but the necessary foundations have already been put in place. With perseverance and focus, the end goals would surely be achieved.

Gabay sa Paggamit ng 10 Halamang Gamot (translated)

Philippine Institute of Traditional and Alternative Health Care

Akapulko

Cassia alata L

Indication: For superficial fungal infections (body ringworm - *Tinea corporis*, athlete's foot - *Tinea pedis, Tinea versicolor*) and scabies

Identification: It is a shrub, about 3 meters or greater in height. Its flowers are yellow in color and are seen at the tip of a branch. The seeds are small and are contained in an elongated seed cover.

Planting: Use stem cuttings approximately 20 cm. in length and with at least 2-3 nodes. Seeds can also be used.

Harvesting and storage: Harvest mature and healthy leaves only.

Preparation: Pound sufficient amounts of fresh leaves.

Administration: Apply the juice on the affected area 1-2 x/day.

Notes: For those allergic to fresh akapulko leaves, use the decoction instead:

Using a clay pot (or any other non-aluminum cookware), boil 1 cup of chopped leaves in 2 cups of water for 15 minutes or until reduced to about 1 cup.

Wash the affected areas with the cooled decoction 1-2 x/day

Ampalaya

Momordica charantia

Indication: Diabetes mellitus (mild, Type 2)

Identification: It is a flowering/fruit-bearing vine. The fruit and leaves are consumed as vegetable.

Planting: Plant the mature seeds.

Harvesting: Harvest the young leaves only.

Make sure that the leaves are clean.

Preparation: Clean the leaves thoroughly and chop them. Measure 1 cup of leaves and 2 cups of water. Using a clay pot (or any other non-aluminum cookware), boil the leaves in low heat for 15 minutes.

Do not cover the pot.

Cool, then strain.

Administration: Consume 1/3 cup 3x/day 30 minutes before meals. The young leaves may also be steamed and then eaten (1/2 cup 2x/day).

Garlic

Allium sativum

Indication: To lower blood cholesterol levels

Identification: Cloves are used to add flavor to food. Garlic is a flowering plant with elongated leaves.

Harvesting and storage: Usually harvested every February and March. Store in a dry and well-ventilated place to prevent rotting and to protect it from being eaten by pests.

Preparation: Saute (with or without oil). Broil. Soak in vinegar for about 30 minutes or blanch with boiled water for about 5 minutes.

Administration: Consume 2 cloves 3x/day after meals.

Note: Make sure to take garlic after meals so as not to cause ulcerations of the stomach.

Guava

Psidium guajava L.

Indication: For cleaning wounds, for treating mouth sores, swelling gums, and dental caries.

Identification: It is a tree, growing up to 4-5 meters in height. Its flowers are white. The fruit is round with small seeds and is eaten fresh.

Planting: Use the seeds for planting.

Care: No special attention is needed for the guava plant to grow.

Harvest/Storage: Harvest young leaves or shoots only.

Make sure that there are no insects or dirt in the leaves.

Preparation: Wash the leaves thoroughly and chop them. Using a clay pot (or any non-aluminum cookware), boil 2 cups of leaves in 4 cups of water for about 15 minutes using low heat. Do not cover the pot. Cool, then strain.

Administration: For wounds:

Wash the wound with the cooled decoction 2x/day.

As oral antiseptic:

Gargle the decoction while it is still lukewarm.

Lagundi

Vitex negundo L

Indication: cough, asthma, fever

Identification: It is a shrub bearing 5 leaves. It is approximately 5 meters in height.

Planting: Prepare stem cuttings bearing at least 3 nodes. Sharpen the edge of the stem, then plant (make sure that 1 node is covered with soil). Place in a well-shaded area for 2 weeks and water the plant. Expose it to sunlight once young leaves have begun to sprout.

Care: Water the plant daily. Remove weeds around the plant.

Harvesting/Storing: Collect the leaves when the plant starts bearing flowers. Harvest young and healthy leaves only. Make sure that enough leaves are left for the plant to survive. Dry the leaves. Store the dried leaves in a plastic bag or in an amber-colored jar and seal tightly.

Preparation: Wash the leaves thoroughly and chop them. Measure 2 cups of water and leaves *(refer below for the appropriate amount of leaves needed per age group).*

Using a clay pot (or any other non-aluminum cookware), boil the leaves for 15 minutes. Do not cover the pot. Cool, then strain.

Age	Amount of leaves	
	fresh	dried
adult	6 tbsp.	4 tbsp.

7-12 years	3 tbsp.	2 tbsp.
2-6 years	1.5 tbsp	1 tbsp.

Administration: For cough and asthma: Divide the decoction into 3 parts. Drink 1 part of the decoction 3x/day

For fever: Drink one part of the decoction every 4 hours.

Note: The *lagundi* plant bearing 1 to 3 leaves should be avoided.

Niyug-niyogan

Quisqualis indica L.

Indication: *Ascaris* infection

Identification: It is a climbing shrub reaching up to 8 meters in height. It bears red, pink, or white flowers. The fruit is small and shaped like a *balimbing* fruit. The seed tastes like coconut meat.

Planting: Prepare stem cuttings at least 20 cm. in length and bearing at least three nodes.

Care: Plant in a shaded area. Transfer to a permanent location after 2-3 weeks. Growth is faster if the plant is exposed to sunlight.

Harvesting/Storing: Harvest the fruit (seed) once it is ripe. The ripened fruits are gold colored. Air dry. Store in a jar and seal tightly.

Preparation: Use only ripe, dry, and freshly opened fruit (seed).

Administration: Consume the seeds 2 hours after supper.

If the desired effect is not yet seen, repeat administration after 1 week using the same dose.

Age	# of seeds needed
Adult	8-10
7-12 years	6-7
6-8 years	5-6
4-5 years	4-5

Note: Adverse effects of *niyug-niyogan* include hiccups, abdominal pain, and diarrhea.

Sambong

Blumea balsamifera (L.) (DC.)

Indication: For edema (acts as a diuretic)

For dissolving urinary tract stones

Identification: It is a shrub approximately 3 meters in height. The leaves are serrated.

Planting: Use plantlets (young plants growing beside the mother plant, with 3 or more leaves). Make sure that the roots of the plantlets have not been detached.

Harvesting and storage: Harvest mature and healthy leaves only. Make sure that enough leaves are left for the plant to survive. Dry the leaves well. Store in a plastic bag or in an amber-colored jar and seal tightly.

Preparation: Wash the leaves well and chop them. Measure 2 cups of water and leaves (*refer below for the appropriate amount of leaves needed per age group).*

Using a clay pot (or any other non-aluminum cookware), boil the leaves in low heat for 15 minutes. Do not cover the pot. Let cool and strain.

Age	Amount of leaves needed	
	fresh	dried
adult	6 tbsp.	4 tbsp.
7-12 yrs	1/2 of adult dose	

Administration: Divide the decoction into 3 parts.

Consume 1 part 3x/day (morning, noon, and night).

Note: Sambong is not used as treatment for urinary tract infections.

Tsaang Gubat

Ehretia microphylla Lam.

Indication: abdominal pain

Identification: It is a shrub about 5 meters in height. The leaves are green, small, and shiny.

Planting: Use stem cuttings approximately 20 cm in height bearing at least 3 nodes. Seeds can also be used.

Care: Water the plant daily. Do not let the weeds grow near the plant.

Harvesting/Storing: Harvest mature and healthy leaves only. Make sure that enough leaves are left for the plant to survive. Air dry. Store the leaves in a plastic bag or in an amber-colored jar and seal tightly.

Preparation: Wash the leaves thoroughly and chop them. Measure 2 cups of water and leaves *(refer below for the amount of leaves needed per age group).*

Using a clay pot (or any other non-aluminum cookware), boil in low heat for 15 minutes. Do not cover the pot. Cool, then strain.

Age	Amount of leaves needed	
	fresh	dried
adults	4 tbsp.	3 tbsp.
7-12 years	1/2 of adult dose	

Administration: Divide the decoction into 2 parts. Drink 1 part every 4 hours.

Note: Can also be used as a gargle to strengthen teeth.

Ulasimang Bato

Pepperomia pellucida

Indication: To lower serum uric acid levels in patients with gout

Identification: A type of grass with heart shaped leaves.

Planting: The plant is widely distributed. The seeds may be used for planting.

Harvesting and storage: Make sure that the source of the leaves is clean (away from dust and automobile exhausts). Harvest healthy leaves only.

Preparation and administration:

As salad: Wash the leaves thoroughly. Prepare 1 1/2 cups of fresh leaves.

Divide into 3 parts and consume 1 part 3x/day.

As decoction: Wash the leaves thoroughly. Prepare 1 1/2 cups of fresh leaves and 2 cups of water. Using a clay pot (or any other non-aluminum cookware), boil in low heat for 15 minutes. Do not cover the pot. Cool, then strain.

Divide the decoction into 3 parts and consume 1 part 3x/day (morning, noon, night) after meals.

Yerba buena

Mentha x cordifolia Opiz ex fresen

Indication: For body aches and pains

Identification: It is a vine, which has a mint odor. The stalk is four sided and is colored green. The leaves are arranged facing each other and are coarse to the touch. They have a crumpled appearance.

Planting: Plant stem cuttings approximately 10-15 cm. in length with at least 3-4 pairs of leaves. Roots will start to develop within 1 week.

Care: Water the plant daily. Remove weeds from the plant's surroundings.

Harvesting and storing: Harvest the mature and healthy leaves only. Make sure that enough leaves are left for the plant to survive. Dry the leaves well. Store in a plastic bag or in a colored jar and seal tightly.

Preparation: Wash the leaves thoroughly and chop them. Measure 2 cups of water and leaves *(refer below for the appropriate amount of leaves per age group).* Using a clay pot (or any other non-aluminum cookware), boil the leaves in low heat for 15 minutes. Do not cover the pot. Cool, then strain.

Age	Amount of leaves needed	
	fresh	dried
adult	6 tbsp.	4 tbsp.

7-12 years	1/2 of adult dose

Administration: Divide the decoction into 3 parts. Consume 1 part 3x/day (morning, noon, night). Fresh leaves may also be minced and applied on the painful area in the body.

COUNTRY REPORT

COUNTRY: Zimbabwe

NAME: Mr. Richard Protaclus Ngwenya

JAMES MOBB IMMUNE ENHANCEMENT

132 Josiah Chinamano Avenue - Harare - Zimbabwe. Tel: (263-4) 725973 Tele/Fax: (263-4) 739832

TREATMENT OF AIDS AND OTHER DEGENERATIVE INFECTIONS WITH MEDICINAL PHYTOCHEMICALS AND NUTRITIONAL SUPPLEMENTS.

P. R. Ngwenya T. MeD. P. BCZ, and M. Gundidza B.Sc., M. Sc., PhD.

ABSTRACT.

Immune disregulation in most chronic infections is associated with a myriad of microorganisms that directly assault the immunological integrity of the body or accelerate the degeneration of the homeostasis of the body. The Kinorfsky HIV Indicator Scale, Somatoscope, viral load analysis and other tests were used to determine the extend of infections. The most common symptoms of a compromised immune system such as loss of weight and appetite, abdominal pains, silky hair, chronic fatigue, candidiasis, arthritis, diarrhea, oedema etc. were treated using herbal antifungals, biofeed infusions, herbal antibacterials, antiparasitics and nutriceuticals. A total of 4800 patients were treated. Results obtained showed that 96.8 per cent of patients recovered from life threatening conditions whilst 3.2 per cent died. The 3.2 per cent who died were mainly cases of patients seeking medication too late. These results clearly indicate that this treatment regimen is very effective as is also indicated by large numbers of patients visiting our clinics daily.

1. OBJECTIVES:

a) To assess the therapeutic benefits of African phytochemicals in the treatment of Immune disregulating diseases.
b) Introduce affordable herbal medicines.
c)Encourage African governments to use local medicinal products to treat *HIV/ AIDS, Cancer, Diabetes, STD's, Arthritis, Hypertension,* and other chronic infections where allopathic medicine fails to give satisfactory benefits.
d)Encourage the use of integrated medical practice in the treatment of HIV/AIDS.

2. INTRODUCTION:

Evasive mycobacterium with mutant pleomorphic behavior in both non-walled captions and DNA structure cultured from the blood of cancer patients ranging from Von Brehmer's syphonospora polymerpha to Lentz's agaragonic bacteria have been studied since the 1850's. Syphilis and other sexually transmitted diseases studies have been followed with inquisitive investigations to determine their etiology. However, very few studies have dealt with the evolution of chronic infections thus encouraging our group to study the phenomena of chronic infection etiology and metabolic disorders. It is our belief that AIDS is a metabolic disease associated with severe dysbiosis from various causative factors. Cold War military biochemical warfare programs have also been

associated with genetically altered immune-suppressing microbes. Poverty and poor hygienic practices in the Third World have also worsened AIDS morbidity.

Most AIDS patients showed severe Adenosine Triphosphate (ATP) depletion caused by serious impaired metabolism and other cellular dysfunctions. Acidosis alters body pH leading to severe acidified states and thereby causing hyperactive nucleated lymphocytes, borne marrow depletion, pancreatic, and liver inflammatory diseases. The bowel secretions such as hydrochloric acid, pepsin and secretin are reduced whilst pancreatic enzymes, amylase, lipase, tyrosine also get depleted thus inhibiting salivation.
The penetration of spermatozoa into somatic mammalian cells also exacerbated the eruption of trophoblast in cancerous AIDS patients as polyamine spermine at higher concentrations interfere with DNA synthesis. (Papadopulos- Eleopulos, Turner and Papadimitiou 92)
Mycoplasma fermentans / incognitos was shown to be the fore front co-factor causing immune collapse (Nicolson and Nasrella 98). The other predisposing virulent strains were *M. penetrans, M. homins, M. genitalis, M. pnemoniae, and M. arthriditis.* Various fungal forms, yeasts, candidiasis, toxoplasmosis, toxic chemicals, water and food contaminants, starvation, sanitary neglect, parasites and bacterium were found to co-actively increase morbidity in African AIDS. Lifestyle influences, alcoholism, stresses, and hopelessness-accelerated mortality.
In order to address these problems we have over years developed an AIDS treatment regimen hereunder described.
A treatment program for immune degenerative diseases was initiated in July 1996 to effectively treat *HIV/ AIDS, Cancer, Rheumatoid Arthritis, Diabetes, Chronic Fatigue Syndrome, Fimbromyalgia, Tuberculosis, PCP, Asthma* and various other symptomatic chronic ailments. Since the introduction of this protocol, 4800 patients were treated. Investigations revealed strong evidence linking multiple strains of these organisms reflecting symptoms and complexes that included *irritable bowel syndrome, Kaposi's sarcoma meningitis, migraines, body odour. memory lose, skin rashes, breathing difficulties, fevers, reactive allergic conditions. Acute damage to parasympathetic and intrinsic nervous cells was depicted to be causing constipation, chronic fatigue, multiple sclerosis, halitosis, tissue inflammatory diseases, septicaemia epithelial/endothelial damage, coagulopathy, anemia, and other predispositions.*

3. MATERIALS AND METHODS:

3.1. Materials

A.C.T 5: For the treatment of fungal infections, cleansing of hepatic toxins, a strong detoxifier with natural immune boosting properties, fights various types of cancer. chronic fatigue syndrome, diabetes, asthma, hypertension, epilepsy, body weakness, and other degenerative diseases. Contains no yeast, colouring and preservatives. Suggested dosage; 1 to 2 capsules twice daily after meals. Patients suffering from acute hepatic infections may double the dose for a week and revert to normal doses as liver damage is cleared.

G. K Seventeen: Eliminates viral, bacterial and minor parasitic infections. Protects patients from inflammatory bowel disease, and other gut infections. Has been used by natives of Africa in the treatment of stomach aches, acidosis, and menstrual disorders.

Messiana: Strong natural anti-parasitic/ laxative. Clears bowel/ intestinal stubborn parasites/worms that cause acute and chronic gastrointestinal complaints, chronic illness, food allergies, auto-immune diseases, mal-absorption, and other systemic diseases that remain unknown. Take one capsule per day twice weekly. This product contains no artificial product sugar, dairy products, colouring, preservatives, yeast, and flavouring. If stomach-burning sensation takes place, take magnesium or a painkiller. WARNING: Not to be used by expecting women.

Profferon: Profferon provides an intestinal defence against hostile microorganisms that destroy normal digestion. Each capsule contains 10 billion IU.s combination of Lactobacillus acidophilus, bulgarius, bifid bacterium bifidum, Bifid bacterium infantis, Streptococcus thermophilus, and Streptococcus faecium in a natural base. These cultures are free of dairy products. Store in a cool dry place; refrigeration is necessary. Works stronger than natural mother's colostrum milk in defence against hostile intestinal bacteria and fungi. Should not be taken in combination with anti-fungal, anti-biotics or anti-parasites as they eliminate friendly flora.

Extra-Plus Vuka Vuka: A potent men's formula extract originating from the ancient Southern African Tshaka-Zulu Vuka Vuka formulation. Activates sexual hyper excitability, stops sexual weakness, impotency, unsatisfactory erection associated with old age. Gives new life to all endocrine glands increases the secretion of hormones & chemical messengers & prevents premature aging. Suitable for men who fail to get an erection and those failing to maintain erection repeatedly. Take one to two capsules per day after meals or when necessary.

Citric Powder: A non-acidic form of natural ascorbatemin C (calcium ascorbate) in combination with high levels of a variety of bioflavonoids, also rose hip and acelora extracts. It is particularly helpful for colds, allergies, hay fever, bruising, anti-fungal varicose veins, nose bleeds, haermorrhoids. Take one teaspoon in hot water or juice two to three times daily.

Excelsa Ointment: Anti-fungal cream for the effective treatment of Kaposi's Sarcoma, Psoriasis, athletes foot (Tinea Pedis), jock itch (Tinea Cruris), and ring worms (Tinea Corporis). Skin fungal disorders, nail diseases, groins and armpits and webs of toes. Relieves itching, cracking, scaling, burning and discomfort that can accompany these conditions. Apply thinly and evenly, two to three times daily. Rub in gently. Continue for at least a week till all signs of infection have disappeared.

Dioscogel: A dioscoginin hormone to reactivate the thymus and thyroid glands. Rejuvenates the whole endocrine functional pathway. Teresterone and progesterone are resuscitated by this plant hormone.
SSI: For the treatment of mouth sores, mouth odour, oral thrush, anti-fungal/ anti-

bacterial. Drop four to five drops on tongue or gurgle 3 times daily. Should be swallowed for systemic ant-fungal relief. SSI is a combination of various essential oils that have been used during the ancient Roman Empire and Egyptian medical practitioners against various hostile oral micro-organisms.

Gundisil: This product is very effective against all types of viruses, bacteria, fungal forms, bacteria, and cancer.

Nutriceuticals: A variety of carefully selected mineral/vitamin supplements as well as wholesome foods are provided to stop starvation.

Anticough remedy: This is a blend of three plant extracts that effectively reduce coughing and is effective against asthma, TB, whooping cough, and other digestive infections.
Green blood: This is a blend of carefully selected vegetable juice with all major nutrients with a pH of around 7.4. This product reduces acidosis.

Aviron: A blend of three plant extracts that correct hormonal imbalances.

3.2. Methods
The program randomly treated and managed Immuno Suppression as an array of various chronic illnesses that are resistant to allopathic medication such as antibiotics/antifungals, penicillin, ketoconazole, tetracycline, ampicillin, sporonox, and other standard therapies. At intervention, some of the study patients had suffered for periods ranging from one year to twenty years without serious improvement. Most patients 95% were previously on allopathic treatment before getting onto this protocol. Those noting changes from standard treatment suffered short-term re-lapses. A greater number patients 75% witnessed and showed drastic improvements within an average of three weeks.
Nutritional Intervention alterations removed sugars, all fermenting products sour milk, yoghurt, cheese, alcohol, and other offending foods. Any increase of high fibre diet, proteins, fat acids and other nutritious foods was encouraged for under nourished patients. Green blood, fresh green vegetables, and juices were found beneficial as unadulterated rich sources of amino acids, enzymes, and other nutrients. Smoking was stopped due to its neurotransmitter termination.
A combination of gastrointestinal/tract and blood cleansers herbal extracts ACT5, GK17, Excelsea capsules, Dioscogel, Mucosin, and Mocrea, a Chinese immuno modulator etc were administered through out therapy. These substances corrected metabolic dysbiosis, shrunk tumors, eliminated pain, sores, reduced viral load, neuropathic conditions and other complexes.
Amino acids, enzymes, and vitamin supplements were introduced to subjects in doses depending on individual depth of infection, weight loss, and other considerations. Messiana was found effective against acute parasitic infections whilst Biosin, Theoplus, and CY110, Chelation Protocols were given at an average of three doses week bypassing oral mal-absorption.
Life Blood Assessment by Somatoscope (LBA) and FBCs were ordered periodically. Severe depletion of WBCs and anemia indicated AIDS's targeting of all blood cells than

previously hypothesized. Anti-depression/stress intervention was instituted to remove the hypothesis of HIV=AIDS= Death myth. All patients were counselled on entry into treatment protocol and when depression was noticed.

4. RESULTS:

Weight lose patients showed a regain of weight within two weeks of intervention as recovery also showed up. Those with silky hair changed to normal hair by week four. Skin rashes showed an improvement within 5-7 days. Diarrhea, itchy skin, oral thrush, memory loss, and other complaints ceased from day 1 to 3 days.

As a non-controlled study evaluating the effectiveness of a combination of African phytochemicals, nutritional supplements and chelates against immuno-comprising diseases, tremendous gains were registered.

Out of the 4 800 study patients 96.8 per cent good recovery was achieved in-patients without serious organ damage liver, pancreas, lungs, brain, endocrine, and other vital organs. Response to medication was poor for patients with acute meningitis. Most patients showed an initial response to therapy within 5 to 7 days reporting a disappearence of some of their symptoms. The death rate was pegged at 3.2 per cent due to severe immune disregulation, functional organ damage, severe weight lose, depression, and starvation.

5. CONCLUSION:

The success of this randomised under funded project gave investigators and patients hope for a healthy recovery from the dreaded so-called HIV/AIDS with an integrated protocol. It has been established from this study that treatment of chronic infections at early stages is more beneficial than late stage interventions. Primary health care facilities could easily assist stop chronic illnesses earlier if this protocol is adopted. Good nutrition, use of condoms and other sanitary considerations must be introduced to maintain anti-reinfection strategies.

6. RECOMMENDATIONS

Scaling up production of natural medicinal products to be implemented as soon as possible.

Funding for land and setting up of a production plant to be given a higher priority.

Setting up of clinics throughout the country to be considered by Ministry of Health.

The investigation team be allowed to treat patients in public hospitals and clinics if government is serious to stop AIDS escalation.

Those medical aid societies should pay for patients on this protocol.

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COUNTRY REPORT

COUNTRY: India

NAME: Mr. Naresh Kumar Satti

Country Report

Dr. Naresh Kumar Satti

India attained freedom on August 15, 1947. India is a peace loving country and is the largest democracy in the World with parliamentary system of Government. It is union of States comprising 28 states and 7 union Territories with a strong centre. Legislature of the union, which is called parliament consists of President of India and two Houses-House of People which is called Lok Sabha and Council of States which is called Rajya Sabha.

Strength of Lok Sabha is 545 members, who are elected by direct election on the basis of adult franchise. The total elective membership of the Lok Sabha is distributed among States in such a way that the ratio between the number of seats allotted, to each state, and population of the State is, as far as practicable, the same for all state. The term of Lok Sabha, unless dissolved, is five years from the date appointed for its first meeting.

Rajya Sabha, which is the upper House, consists of 245 members. Of these, 233 members represent the States and the Union Territories. 12 members are nominated by the President, from amongst persons having special knowledge or practical experience in respect of such matters as literature, science, art and social service.

There is a council of ministers headed by the Prime Minister. The Prime Minister is appointed by the President, who also appoints other ministers on the advice of Prime Minister. The council is collectively responsible to the Lok Sabha.

The system of Government in States closely resembles that of Union. State executive consists of Governor, who is appointed by the President of India and council of ministers with chief Minister as its head who runs the State administration, regarding the subjects of the State as per schedule of the

constitution. Union Territories are administrated by the President, through an administrator appointed by him.

Constitution of India is secular. It guarantees all the basic fundamental rights to its countrymen, irrespective of the religion or faith. It also guarantees independent judiciary, the Supreme Court is the apex court.

Election Commission, Comptroller and Auditor General and Public Service commission further the provisions of the constitution.

As said, India is vast country with and area of Thirty two Lakhs, eighty seven thousand, two hundred sixty three square Kms and population of more than one hundred crores. India has land frontier of about fifteen thousand two hundred Kms. The total length of the coastline of the mainland, Lakshadweep islands, Andaman & Nicobar islands is seven thousand five hundred sixteen Kms.

India has common border with Afghanistan and Pakistan on the north-west. China, Bhutan and Nepal on the north. Myannar on the east and Bangladesh to the east of West Bengal.

Climate of my country is tropical monsoon type. There are four seasons.

Winter : from January to February

Summer : from March to May

Rainy : from June to September

Post monsoon : from October to December

India's climate is affected by two seasonal winds-the north east monsoon commonly known as winter monsoon, blows from land to sea, whereas summer monsoon blows from sea to land after crossing the Indian Ocean, the Arabian sea and the bay of Bengal.

With a wide range of climatic conditions, India has a rich and varied vegetation. Nature has been very kind to provide my country all the natural resources. There are Himalayas, a number of rivers, lakes, thick forests, minerals and all kinds of flora and fauna.

India is very rich in flora. About forty nine thousand species of plants have been identified by the Botanical Survey of India. The total plant wealth of the country includes not only the useful large flowered plants, including flowering shrubs, but also a large number of non-flowering plants like ferns, algae and fungi.

My country has a great variety of fauna numbering eighty one thousand two hundred fifty on species.

India is an agriculture country. India has also a long and distinguished tradition in science from the accomplishments of ancient times to great achievements during 20th century. In the past four decades, an infrastructure and capability largely commensurate with meeting national needs has been created, minimizing our dependence on other countries. A large range of industries from small to most sophisticated have been established covering wide range of utilities, services and goods.

A strong science and technology infrastructure has been established in the country. This covers a chain of national laboratories, specialized centres, various R&D and academic institutions and training centres.

My country maintains harmony with all. It persues the path of non-violence and peaceful co-existence. India has been practicing and preaching these principles of non-violence and peaceful co-existence. Since time immenorial, Lord Budha, Mahavir, Ram Tirth, Vivekanand, Yoganand, Guru Nanak Sant Kabir and Mahatama Gandhi travelled different countries of the World and preached these Godly message to the World.

My country's national flag is a horizontal tricolor of deep saffron at the top, white in the middle and dark green at the bottom in equal proportion. In the centre of the white band is a navy blue wheel having 24 spokes.

In our country's emblem, three lions are visible, the wheel appears in relief in the centre of the abacus with a bull on the right and a horse on the left and outlines of other wheels on extreme right and left.

We have a national anthem. The magnificent tiger is our national animal and peacock is our national bird.

There are number of places in India which tempt World tourist, some of these are Ajanta, Elora, Elephanta, Kanhavi, Karla caves, Taj Mahal and Kashmir valley.

COUNTRY REPORT

COUNTRY: Sri Lanka

NAME: Dr. Jayantha Wijayabandara

Workshop on Research Strategies on Medicinal and Aromatic Plants in Bangkok , Thailand from 14 to 18 August 2000

COUNTRY REPORT – SRI LANKA

BY

Dr. Jayantha Wijayabandara
Senior Lecturer in Pharmacy
Department of Pharmacology and Pharmacy
Faculty of Medicine,
University of Colombo
Colombo 08,
Sri Lanka.

1. INTRODUCTION

1.1 General Introduction

Sri Lanka is a tropical continental island located in the Indian Ocean. The total land-area of geographical entity of Sri Lanka is approximately 65,600 Km^2.

Medicinal plants are widely used in Sri Lanka in Ayurvedic system of medicine which is one of the systems of medicine recognized by the Government of Sri Lanka.

1.2 Biodiversity :

There are about 1,414 species of medicinal plants that have been used for treating and preventing diseases traditionally. Ayurveda serves the health needs of 70% of 18.6 million of the country's population. Of the 1,414 medicinal plants species. 189 species or 13.3% are endemic to Sri Lanka including 79 species which are believed to be threatened in the wild. 208 plants are commonly used in the Ayurvedic system of medicine and of them about 50 species are used in large quantities.

1.3 Traditional Medicine in Sri Lanka today

The traditional system of medicine has been practiced in Sri Lanka for over 3000 years. According to the Ayurveda Act of No. 31 of 1961 of the government of Ceylon Ayurveda includes the Siddha, Unani and Desiya Chikitsa systems of medicine and Surgery.

Besides the traditional medical practices which are recorded in ancient manuscripts , written on palm leaves by practitioners of Ayurveda and in other classics, there is also a great deal of scattered knowledge in folk medicine which utilizes medicinal plants of different localities.

The cheap and effective traditional remedies that existed and still used in Sri Lanka is an economic and sound approach for the health care to the poor and to those in the remote areas of the country.

2. PRODUCTIN OF HERBAL MEDICINAL REMEDIES.

Herbal drugs are being produced by several establishments on a commercial scale. At present , Government (eg. Ayurvedic Drug Corporation of Sri Lanka) as well as private (eg. Link Natural Products (pvt.) Limited, Hettigoda Group of companies) organizations are producing herbal medicinal products. Most of these medicines are now exported to UK, USA, Germany, and India.

3. CULTIVATION OF MEDICINAL PLANTS.

With a some exceptions, many of the medicinal plants used by the local industry are harvested from the wild. As a result of unmanaged harvesting and habitat destruction, and the lack of organized cultivation system, many plant species are threatened. It is therefore vital that systematic cultivation of these plants be introduced in order to conserve the biodiversity and protect endangered species.

4. SRI LANKA CONSERVATION AND SUSTAINABLE USE OF MEDICINAL PLANT PROJECT

The medicinal plants conservation project is an activity of the Ministry of Health and Indigenous Medicine of Sri Lanka. The main objective of the project is to conserve the significant medicinal plants, their habitats, species and genomes and promote their sustainable use in Sri Lanka. The project seeks to achieve this through the establishment of medicinal plant conservation areas where there is a collection of medicinal plants from the wild. The following sites have already been selected for this purpose.

a. Bibile
b. Retigala
c. Naula
d. Rajawaka and
e. Kanneliya

Activities have already begun in all sites. The project strives to increase the populations of medicinal plant species by,

a. Improving significantly the conservation and sustainable use of medicinal plants in selected sites through *in situ* conservation.
b. Promoting *ex situ* cultivation of medicinal plants species; and
c. Improving the knowledge base, institutional capacity and awareness.

Other activities of this project consist of a Socio –economic Survey, ethnobotanical survey and a resource inventory.

5. RESEARCH ACTIVITIES.

Research activities have been carried out by the following institutions on various aspects of medicinal plants and herbal medicinal remedies.

- University of Sri Jayewardanepura
- University of Peradeniya
- University of Colombo
- Industrial Technology Institute (formerly CISIR)

- Bandaranaike Memorial Ayurvedic Research Institute (BMARI)
- Link National Products (pvt;) Limited.

Areas of research in medicinal plants and herbal medicinal remedies done at these institutions include:

- evaluation of efficacy and mechanisms of action of traditional herbal preparations
- formulation of criteria for the quality control and standardization of Ayurvedic drugs and drug preparations.
- analysis of medicinal plants on compounds having biological properties through activity directed studies.
- structure elucidation of medicinal plant constituents.
- formulation of new products.
- clinical studies on medicinal plants.

a) **Some Research Achievements of the University of Sri Jayewardanepura .**

Significant research work. particularly in immunomodulation and drug standardization. has been carried out by the Department of Chemistry of University of Jayewardanepura in collaboration with Department of Pharmacognosy of the University of Utrecht, The Netherlands.

- Immuno modulatory properties of medicinal plants leading to discovery of immunotheraputic agents.
  In the primary health care of third world countries , immune- based diseases are successfully treated with traditional medicines.
- For the first time in Sri Lanka indigenous drugs were evaluated on their therapeutic efficacy on immune-based diseases. - a mammoth task !
- Formulation of criteria for the quality control and standardization of Ayuvredic drugs and drug preparation procedures (i.e. arishta and asawa preparation)
- Pharmacognostical studies on medicinal plants.
- evaluation of efficacy and mechanisms of action of traditional herbal preparations.
- structure elucidation of medicinal plants constituents.

b) **Some Research Achievements of the University of Peradeniya.**

- Structure elucidation of medicinal plants constituents.
- Anti-bacterial , anti-fungal and insecticidal properties of medicinal plant constituents.

c) **Some Research Achievements of the University of colombo.**

- Structure elucidation of medicinal plant constituents
- Biotechnological methods for mass propagation of medicinal plants

- Pharmacological studies of medicinal plant extracts.

d) **Some Research Achievements of the Industrial Technology Institute.**

- Seasonal variation of biologically active plant constituents.
- Agronomical studies
- Preparation of value added products.
- Structure elecidation of plant constituents.
- Research studies to work out pilot plant scale extraction procedures
- Formulation of medicinal plant based products

e) **Some Research Achievements of BMARI and Ayurveda Hospital Borella.**

- Clinical research on Ayurvedic preparations
- Quality control of Ayurvedic preparations.
- Agronomical studies.

f) **Some Research achievements of Link Natural Products (pvt.) Ltd;**

- Development of new formulations based on medicinal plants
- Utilization of most modern technology in the manufacture of Ayurvedic drugs.
- Quality control of Ayurvedic products

6. INTELLECTUAL PROPERTY RIGHTS (IPR) OF MEDICINAL PLANTS

Preparation of legal regime to safeguard traditional knowledge relating to the use of medicinal plants is now being done by the Ministry of Health and Indigenous Medicine.

7. AYURVEDIC DRUGS, COSMETICS AND DEVICES ACT

Ministry of Health and Indigenous Medicine has prepared the law to amend the Ayurvedic Act No. 31 of 1961. In addition , the Ministry has introduced a new quality control law titled Ayurvedic Drugs , Cosmetics and Devices Act which provides a comprehensive and effective legal framework for the preservation and development of the indigenous medicine sector.

8. TRAINING

Training of Ayurvedic doctors are done by the University of Colombo (Institute of Indigenous Medicine) and the University of Kelaniya (Gampaha Wickramarachchi Ayurveda college). Post graduated course are conducted at the National Institute of Traditional Medicine.

9. FINAL REMARKS

Sri Lanka is a country rich in medicinal plant biodiversity which could be treated as a rich source for the search for new drugs. Also , it is recognized that only a small portion of this diversity has been studied for its medicinal potential in terms of modern science. Hence there is a sense of urgency in studying these medicinal plants in order to unlock their pharmaceutical and medicinal secrets.

There is a tremendous need of funds for the evaluation of activity of medicinal plants leading to new drug discovery. To this end, fruitful collaborative efforts are needed. I hope that the ICS and UNIDO would support collaborative research among her Asian countries present at this meeting.

ACKNOWLEDGEMENTS

I would like to thank ICS and UNIDO for giving me this opportunity to upgrade my knowledge and to develop contacts.

COUNTRY REPORT

COUNTRY: Thailand

NAME: Dr. Krisana Pootakham

COUNTRY REPORT

PRESENT TO THE INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY, ICS-UNIDO AND GOVERNMENT PHARMACEUTICAL ORGANIZATION AT A WORKSHOP ON RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS. BANGKOK, THAILAND. AUGUST 14-18,2000

BY

DR. KRISANA POOTAKHAM

FACULTY OF PHARMACY

CHIANG MAI UNIVERSITY

THAILAND

Blood-glucose lowering(Hypoglycemic)activity of some Thai medicinal plants

Diabetes mellitus(DM) is the disorder of body metabolism which indicates by high blood – glucose level(more than 160 mg%). There are 3 types of DM recognized by the World Health Organization which are insulin-dependent diabetes mellitus(IDDM), non-insulin-dependent diabetes mellitus (NIDDM) and malnutrition-related diabetes mellitus (MRDM). More than 30 million people throughout the world suffer from this disease. Life expectancy may be halved by this disease, especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable. NIDDM is a major health problem in populations undergoing modernization of life-style.

Herbal drugs from plants (more than 1,000 species) have been long used to cure DM in developing countries including Thailand. Reports from hospitals in Thailand revealed that more than 80% of DM patients used herbal drugs to treat DM before going to the hospital and/or simultaneously with the modern treatment and 50% were confident to used herbal drugs.

Plants traditionally used in treatment of DM in Thailand.

Allium sativum Linn. (Alliaceae)

Allium cepa Linn. (Alliaceae)

Angiopteris evecta Hoffm (Marattilaceae)

Carissa carandas Linn. (Apocynaceae)

Coccinia grandis Voigt. (Cucurbitaceae)

Cymbopogon citratus Stapf. (Graminae)

*Ganoderma lucidum*Karsten(Polyporaceae)

Lagerstroemia speciosa Pers.(Lythraceae)

Momordica charantia Linn.(Cucurbitaceae)- many varieties

Momordica balsamina L.(Cucurbitaceae)

Morinda citrifolia Linn. (Rubiaceae)

Morinda elliptica Ridl. (Rubiaceae)

Morus alba Linn. (Moraceae)

Ocimum canum Sims. (Labiatae)

Orthosiphon grandiflorus Bolding (Labiatae)

Pnadanus odorus (Pandanaceae)

Panax ginseng C.A. Meyer (Araliaceae)

Phyllanthus urinaria Linn.

(Euphorbiaceae)

Phyllanthus amarus Schum & Thonn. (Euphorbiaceae)

Piper sarmentosum Roxb. (Piperaceae)

Piper auranticum Miq. (Piperaceae)

Psidium guajav Linn.(Myrtaceae)

Solanum sanitwongsei Craib.(Solanaceae)

Solanum tribolatum Linn. .(Solanaceae)

Tectona grandis Linn.(Verbenaceae)

Trigonella foenum-graecum Linn.(Fabaceae)

Tinospora crispa (L.) Miers ex Hook f. *et*

Thoms. (Menispermaceae)

So it is very interesting to scientifically investigate Thai medicinal plants traditionally used as hypoglycemic agent.

Plants choosing for hypoglycemic activity screening.

Angiopteris evecta Hoffm (Marattilaceae), stem

Carissa carandas Linn. (Apocynaceae), leaves

Coccinia grandis Voigt. (Cucurbitaceae), stem

Cymbopogon citratus Stapf. (Graminae),stem

Gymnema inodorum Decne (Asclepiadaceae), leaves

Gymnostemma pentaphyllum Makino(Cucurbitaceae), whole plant

Momordica charantia Linn. (Cucurbitaceae)- fruits and flowering tops of many varieties

Momordica balsamina L. (Cucurbitaceae)- flowering tops

Morinda citrifolia Linn. (Rubiaceae), mature fruits

Morinda elliptica Ridl. (Rubiaceae), root

Ocimum canum Sims. (Labiatae), mucilage from fruits

Phyllanthus urinaria Linn. (Euphorbiaceae), whole plant

Phyllanthus amarus Schum & Thonn. (Euphorbiaceae), whole plant

Piper sarmentosum Roxb. (Piperaceae), whole plant

Piper auranticum Miq. (Piperaceae), whole plant

Solanum sanitwongsei Craib.(Solanaceae), fruits

Tinospora crispa (L.) Miers ex Hook f. *et* Thoms.

(Menispermaceae), stem

Tinospora cordifolia Miers (Menispermaceae), stem

Methodology

1. Preparation of crude extract of medicinal plants- water extract in the form of spray dry powder.
2. Glucose tolerance test in normal rat(Wistar)
   - -Blood glucose level in fasting rats was measured by Glucometer® using Glucotide®strip.
   - -Crude extract of each plant was administered orally at the dose of 1,2,3,4 & 5 g/kg body weight.
   - -After 30 min, glucose at the dose of 2 g/kg body weight was administered orally and intraperitoneally separately in each group of rats.
   - -Blood glucose level was measured at 30 min interval to 180 min.
3. Hypoglycemic activity of crude extract in alloxan-induced rats
   - -Blood glucose level in fasting rats was measured by Glucometer® using Glucotide® strip.
   - -Crude extract of each plant was administered orally at the dose of 1,2,3,4 & 5 g/kg body weight.

-Blood glucose level was measured at 30 min interval to 180 min.

Results

1 .As a single dose administration with a maximum dose at 3-5 g/kg body weight, none of the crude extract showed promising hypoglycemic activity in normal rats.

2. Plants showed hypoglycemic activity in alloxan-induced rats.

<u>At the dose of 1 g/kg body weight.</u>

Gymnema inodorum Decne

Momordica charantia L. –All varieties

Momordica balsamina L.

Morinda citrifolia Linn.

Phyllanthus urinaria Linn.

Piper auranticum Miq.

<u>At the dose 2 g/kg body weight</u>

Gymnostemma pentaphyllum Makino

3. Other plants showed doubtfully hypoglycemic activity in alloxan-induced rats and need to be further studied.

However, multiple dose administration should be done with these crude extracts because the mechanism of action may be due to induction of insulin and/or stimulation of glucose utilization.

As development of a new drug involved many steps and also time consuming process, thus it is suggested to use these plants as food supplement to control blood-glucose level in simultaneous with the modern treatment.

COUNTRY REPORT

COUNTRY: Thailand

NAME: Dr. Sanan Subhadhirasakul

Country report

Southern Thailand and Faculty of Pharmaceutical Sciences

Sanan Subhadhirasakul, Ph.D.

Southern Thailand, located on the Malay Peninsula, has a characteristic of the long, narrow land strip surrounded by Indian Ocean in the west and Pacific Ocean in the east. It has good combination of various types of topography, including lower plains, plateaus, hills and mountains. Along the length of land strip are the mountain ranges, from Tanowsri range in the upper part to Banthad and San Kalakiri ranges in the lower. These mountain ranges, set in the middle of land strip, divide the land into two coastal areas, western and eastern coasts of Southern Thailand. The local climate is highly influenced by regional monsoons from both directions, e.g., the Southwestern and Northeastern Monsoons. These contribute to the area's reputation as the rainiest part of the country, with eight month long rainy season, and provides Southern Thailand its rich herbal and plant flora and high biodiversity.

The Faculty of Pharmaceutical Sciences, Prince of Songkla University, is the only pharmacy school in the southern region. Its organization is composed of five departments, i.e., Department of Clinical Pharmacy, Department of Pharmaceutical Chemistry, Department of Pharmacognosy and Pharmaceutical Botany, Department of Pharmacy Administration, and Department of Pharmaceutical Technology. Also included as one department is the Division of

Pharmaceutical Research and Service, whose main tasks are to facilitate the research works in the school, and to provide academic services to the community.

As part of the organization, the Department of Pharmacognosy and Pharmaceutical Botany has been taking its prime responsibility in the research areas involving medicinal and aromatic plants. The research projects currently conducted in the department can be categorized into three main areas;

1. **Phytochemical studies**: This research area includes the search for pharmacologically and biologically active constituents found in medicinal plants, starting from selection of leading medicinal plants, taxonomic identification, separation and purification of the compounds, and biological activity determination of the plants and purified samples.

2. **Formulation and standardization of herbal preparations**: The research area involves preparing of herbal monographs and formulating the herbal preparations of medicinal plants of interest. The researches in this area are held under good collaboration with the Department of Pharmaceutical Technology to achieve the desired pharmaceutical preparations with good quality and high stability.

3. **Tissue cultures**: The investigations in the area of medicinal plant tissue cultures conducted in the department are primarily aimed to increase the yield of active constituents from targeted plants, and/or to induce micropropagation of medicinal plants of interest.

Moreover, the department also provides the academic services in different areas relating to medicinal plants. These include:

1. **Herbal medicine manufacturing**: The production of selected herbal medicines, e.g., *Andrographis paniculata* Capsules, *Tinospora crispa* Capsules, *Curcuma domestica* Capsules, is conducted in the department. Although still in small scale, the quality control in manufacturing processes, including assay of active ingredients in each preparation, are strictly practiced to achieve high standard in quality assurance.

2. **Identification and standardization of medicinal herbs**: The department offers the service in identification and standardization of indigenous medicinal herbs of interest to the community. This service has been originated very recently, and still in its developing process.

3. **Detection of steroidal anti-inflammatory drugs in herbal medicines**: The service is provided to those who need to determine whether the herbal preparations of interest, mostly traditional medicines, are contaminated with steroidal anti-inflammatory drugs, which, in most cases, are voluntarily added.

Faculty members of Department of Pharmacognosy and Pharmaceutical Botany and their researches of interest.

1. Pharkphoom Panichayupakaranant, Ph.D. (Department Head)

Research area:-

- Medicinal plant tissue cultures.
- Formulation, analyses and evaluation of herbal medicines.
- Antimicrobials from medicinal plants.
- Anticandidiasis substances from medicinal plants and semisynthesis.
- Antispasmodic substances.

2. Assoc. Prof. Sanan Subhadhirasakul, Ph.D.

Research area:-

- Indole alkaloids from medicinal plants.
- Biologically active constituents from medicinal plants;
  - Immunomodulating agents.
  - Antimicrobial substances.
  - Antinociceptive, antipyretic and anti-inflammatory substances.
  - Biologically active compounds from mushroom.
- Formulation and standardization of herbal preparations.

3. Assist. Prof. Niwat Kaewpradub, Ph.D.

Research area:-

- Determination of biologically active compounds from natural products, particularly medicinal plants.
- Chemistry of natural products.
- Phytochemotaxonomy.
- Standardization of medicinal herbs, indigenous drugs and phytomedicines.

4. Anuchit Plubrukarn, Ph.D.

Research area:-

- Marine natural products.
- Syntheses, both semi- and total syntheses, of natural products.

5. Assoc. Prof. Arunporn Itharat

Research area:-

- Phytochemistry.
- Antioxidant and anticancer from medicinal plants.
- Formulation of herbal medicines.

etc.

ANNEXE C

COPIES OF CERTIFICATES PRESENTED TO PARTICIPANTS OF THE TRAINING COURSE

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

CERTIFICATE OF PARTICIPATION

This is to certify that

ASSOC. PROF. DR. SURAPOTE WONGYAI

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

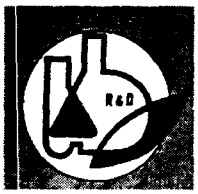
held at the Research and Development Institute,
The Government Pharmaceutical Organization, Thailand
from 14-18 August, 2000
has this day been awarded a certificate of participation

18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS



CERTIFICATE OF PARTICIPATION

This is to certify that

MRS. VANIDA CHANTEPTAWAN

having attented the National Workshop on

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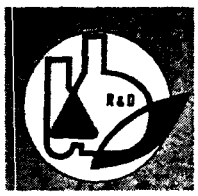

CERTIFICATE OF PARTICIPATION

This is to certify that

MR. SANYA HOKPUTSA

having attented the National Workshop on

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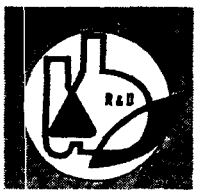

CERTIFICATE OF PARTICIPATION

This is to certify that

DR. FARZANA SHAHEEN

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DR. RITCHE MANOS HAO

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CERTIFICATE OF PARTICIPATION

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DR. RICHARD PROTACIUS NGWENYA

having attented the National Workshop on

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DR. NARESH KUMAR SATTI

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R&D, GPO

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CERTIFICATE OF PARTICIPATION

This is to certify that

DR. MARIANE NGOULLA

having attented the National Workshop on

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ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

CERTIFICATE OF PARTICIPATION

This is to certify that

DR. JAYANTHA WIJAYABANDARA

having attented the National Workshop on

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R&D, GPO

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CERTIFICATE OF PARTICIPATION

This is to certify that

ASSOC. PROF. DR. SANAN SUBHADHIRASAKUL

having attented the National Workshop on

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CERTIFICATE OF PARTICIPATION

This is to certify that

ASSOC. PROF. DR. KRISANA POOTAKHAM

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ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

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CERTIFICATE OF PARTICIPATION

This is to certify that

MS. ABEBA BERHANE KASSAYE

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

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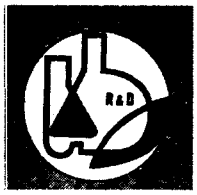
18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS



CERTIFICATE OF PARTICIPATION

This is to certify that

MS. BERNA ELYA

having attented the National Workshop on

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18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

CERTIFICATE OF PARTICIPATION

This is to certify that

MS. MONEKHAM SENGSAVANG

having attented the National Workshop on

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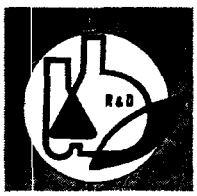
from 14-18 August, 2000
has this day been awarded a certificate of participation

18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS



CERTIFICATE OF PARTICIPATION

This is to certify that

MR. KOK SEONG LIM

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

held at the Research and Development Institute,
The Government Pharmaceutical Organization, Thailand
from 14-18 August, 2000
has this day been awarded a certificate of participation

18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

CERTIFICATE OF PARTICIPATION

This is to certify that

DR. CHIRANJIVI REGMI

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

held at the Research and Development Institute,
The Government Pharmaceutical Organization, Thailand
from 14-18 August, 2000
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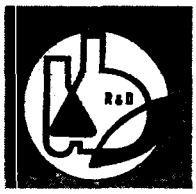
18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS



CERTIFICATE OF PARTICIPATION

This is to certify that

DR. CHENG SUN KAING

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

held at the Research and Development Institute,
The Government Pharmaceutical Organization, Thailand
from 14-18 August, 2000
has this day been awarded a certificate of participation

18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

CERTIFICATE OF PARTICIPATION

This is to certify that

MR. PHURPA WANGCHUK

having attented the National Workshop on

RESEARCH STRATEGIES ON MEDICINAL AND AROMATIC PLANTS

held at the Research and Development Institute,
The Government Pharmaceutical Organization, Thailand
from 14-18 August, 2000
has this day been awarded a certificate of participation

18.08.2000

Dr. K. Vasisht
ICS-UNIDO

Dr. K. Kraisintu
R&D, GPO

ANNEXE D

PHOTOGRAPHS OF ACTIVITIES DURING THE TRAINING COURSE

Fig.1 Group picture taken outside the Research and Development Institute, Government Pharmaceutical Organization, Bangkok

Fig.2 Participants and guests enjoying themselves at the welcome reception

Fig.3 Dr Krisana Kraisintu, Head of R&D Institute, GPO greeting participants at the welcome reception

Fig.4 Dr Krisana Kraisintu presenting a souvenir to guest lecturer Professor Norio Aimi after the lecture

Fig.5 Participants during a lecture

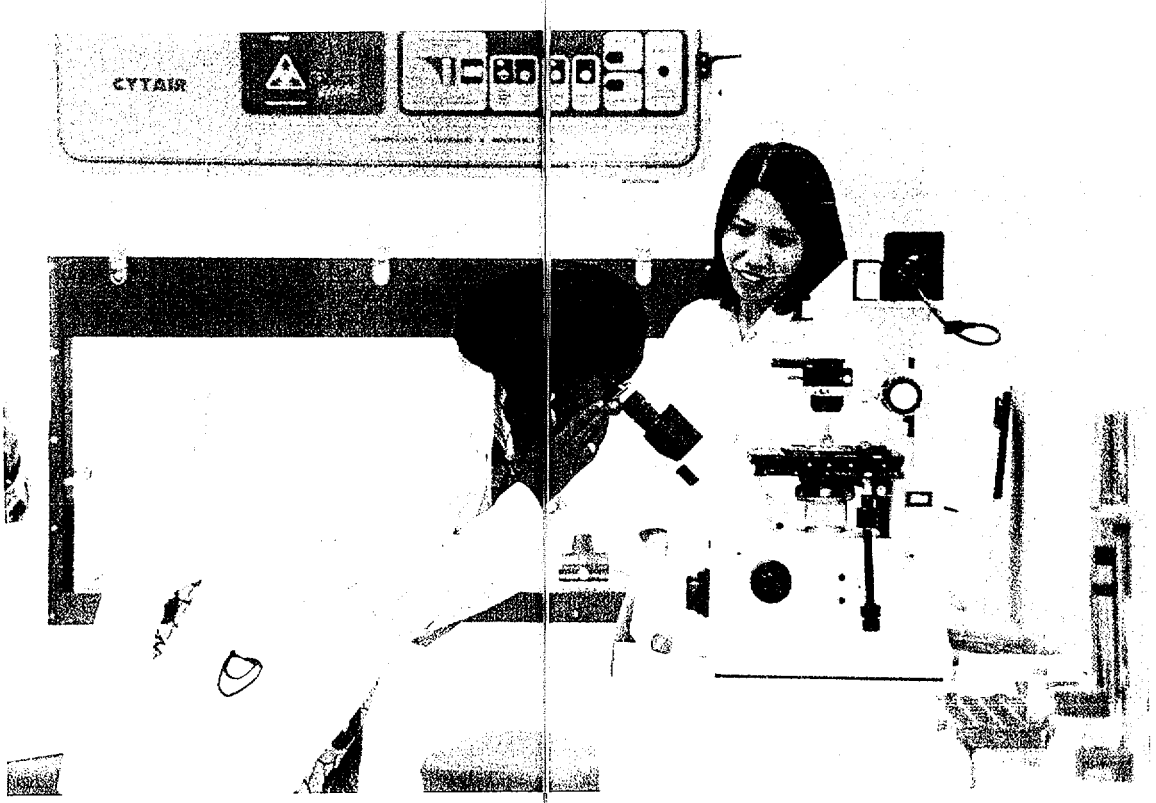


Fig.6 Staff of R&D Institute, GPO demonstrating to a participant during the workshop on Bioactivity screening

Fig.7 Staff of R&D Institute, GPO and participants during the workshop on Extraction and Purification of Medicinal Plants

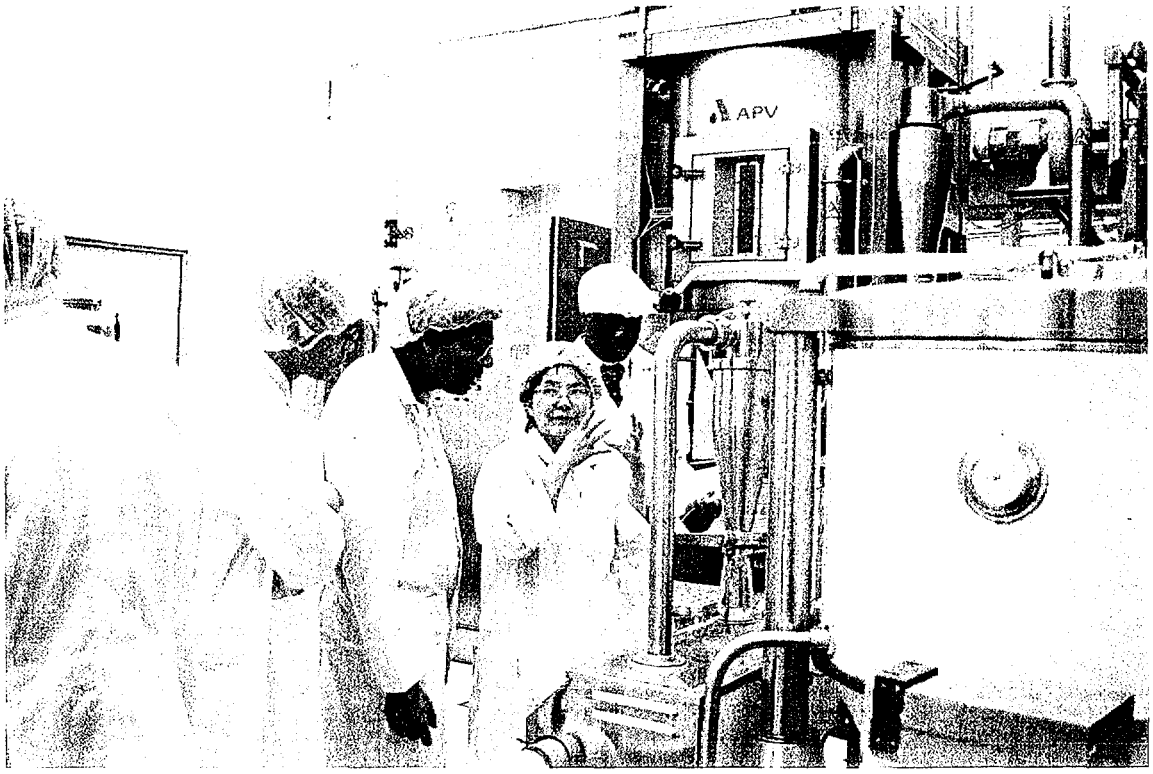


Fig.8 Participants being shown round the production unit of the R&D Institute

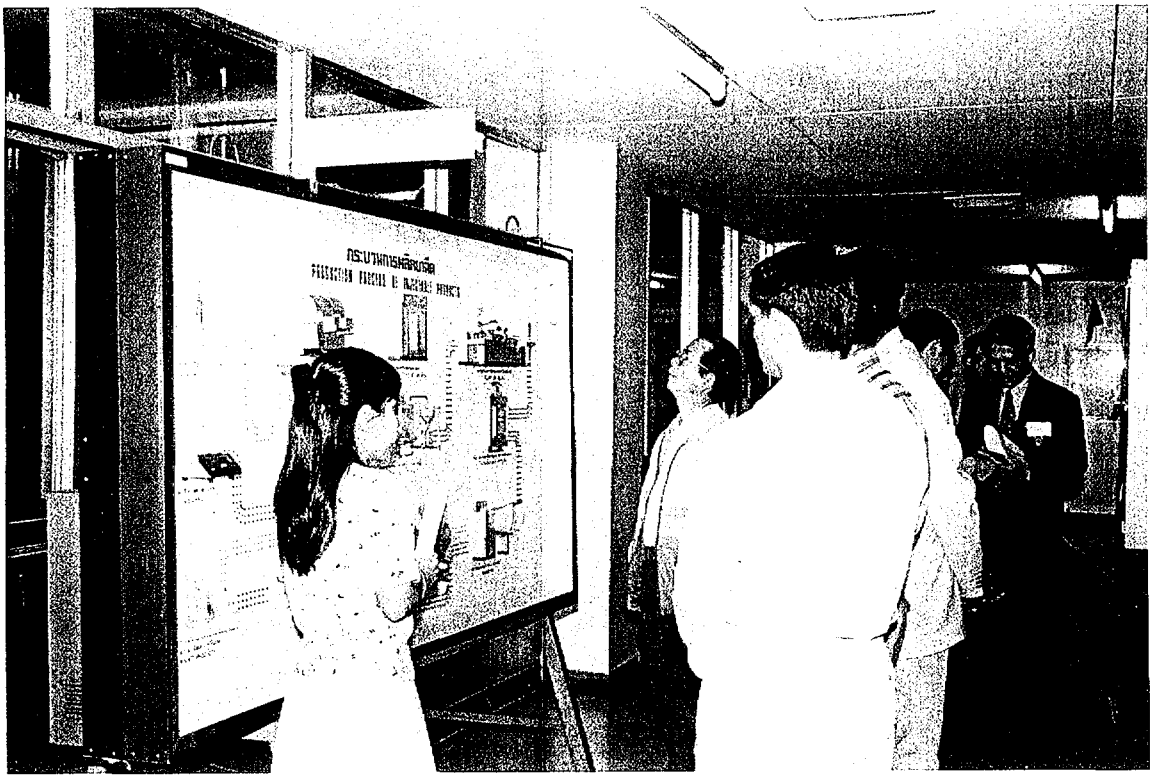


Fig.9 Participants during tour of the production unit of the GPO

Fig.10 Guest lecturer, Professor S.S. Handa and ICS-UNIDO scientific advisor, Dr Karan Vasisht being interviewed by a local news reporter

Fig.11 Group photo taken outside the rice bran oil in Singhaburi

Fig.12 Participants amid the ruins of the old capital, Ayutthaya

Fig.13 Dr Krisana Kraisintu presenting a gift to Dr Karan Vasisht, scientific advisor to ICS-UNIDO

ANNEXE E

LOCAL NEWS REPORT OF THE TRAINING COURSE

อภ.ดัน ICS ถกวิชาการสมุนไพร

นพ.กฤษฎา มนูญวงศ์ ผู้อำนวยการองค์การเภสัชกรรม เปิดเผยว่า สถาบันวิจัยและพัฒนาองค์การเภสัชกรรม ร่วมกับศูนย์วิทยาศาสตร์และเทคโนโลยีชั้นสูงระหว่างประเทศหรือ ICS-UNIDO จัดอบรมวิชาการด้านสมุนไพรร่วมกับนักวิชาการจาก 15 ประเทศ ได้แก่ ไทย ไนจีเรีย ซิมบับเว เอธิโอเปีย ปากีสถาน อินเดีย ศรีลังกา ภูฏาน เนปาล พม่า ลาว เขมร เวียดนาม อินโดนีเซีย และฟิลิปปินส์ เพื่อเป็นการแลกเปลี่ยนความรู้ทางด้านการวิจัยสมุนไพรกับนานาประเทศ ณ ห้องประชุมชั้น 3 สถาบันวิจัยและพัฒนา องค์การเภสัชกรรม ซึ่งจะมีการนำเสนอผลงานวิจัยเรื่องการสกัดสารบริสุทธิ์จากสมุนไพร การพิสูจน์โครงสร้างของสารบริสุทธิ์ที่สกัดออกมาโดยผู้เชี่ยวชาญจากประเทศญี่ปุ่น การวิเคราะห์สารสำคัญจากยอระเหิด การทดสอบความเป็นพิษของสมุนไพรต่อเซลล์สัตว์และเซลล์ปรกติ รวมทั้งการพิสูจน์ฤทธิ์ของสมุนไพรที่สกัดออกมาว่า สามารถจะป้องกันเซลล์และสัตว์ได้หรือไม่ ตลอดจนการบรรยายเรื่องการควบคุมคุณภาพสมุนไพร การอบรมการบันทึกข้อมูลการใช้สมุนไพรสั่งแต่สมัยโบราณจนถึงปัจจุบัน และการรายงานการปฏิบัติการด้านสมุนไพรต่างๆของแต่ละประเทศ.

ไทยรัฐ 14 ธ.ค. 43 หน้า 12

อบรมวิชาการด้านสมุนไพร...

น.พ.กฤษฎา มนูญวงศ์ ผู้อำนวยการองค์การเภสัชกรรม เปิดเผยว่า สถาบันวิจัยและพัฒนาองค์การเภสัชกรรม ร่วมกับ ICS-UNIDO จัดอบรมวิชาการระหว่างวันที่ 14-18 สิงหาคม ที่ห้องประชุมชั้น 3 ของสถาบัน เพื่อแลกเปลี่ยนความรู้ทางด้านการวิจัยสมุนไพรกับนานาประเทศ มี 15 ประเทศเข้าร่วม คือ ไทย ไนจีเรีย ซิมบับเว เอธิโอเปีย ปากีสถาน อินเดีย ศรีลังกา ภูฏาน เนปาล พม่า ลาว เขมร เวียดนาม อินโดนีเซียและฟิลิปปินส์

เดลินิวส์ 15 ธ.ค. 43 หน้า 30

ข่าวจากการแพร่แผ่นดินของประชาชีวันนี้ เมื่อวันที่ 10 ส.ค. 43